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The Journal of Laboratory and Clinical Medicine

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CLINICAL AND EXPERIMENTAL

THE BEHAVIOR OF BACTERIOPHAGE IN SUGAR MEDIA*

BY EMIL WEISS, M.D., CHICAGO, ILL.

SEVERAL authors studied the influence of H-ion concentration on the reaction of the bacteriophage. Our attention was especially attracted by papers, which reported the changes on phage, due to the presence of fermentable sugars in the medium. Asheshov[1] found that certain races of bacteriophage appear more active, if the medium contains glucose; he attributes the increased activity of phage to the changes in the reaction of the medium. Seiser[2] was able to confirm the findings of Asheshov on all races of bacteriophage with which he worked. D'Herelle[3] claims that the addition of glucose causes acidification of the medium and ultimately leads to the destruction of phage; these changes occur only if the sugar is fermentable. He does not mention the concentration of glucose used in his experiments. Eliava and Pozerski[4] found the optimal range of the H-ion concentration for the phage between P_H 2.5 and P_H 8.5. Scheidegger[5] found that the phage at P_H 4.5 remain inactive but when transferred to a slightly alkaline medium, they again show lytic activity; he found that bacteriolysis occurs at P_H 6.5. Gratia[6] found the reaction of the medium at P_H 8.5 as most favorable for bacteriophagy, although lysis occurs at P_H 6.8. Da Costa Cruz[7] found lysis occurring in Martin's broth at P_H 6.5. D'Herelle recommends the ordinary peptone broth at P_H 7.8 as the best medium for the phagic reaction. According to D'Herelle the different results of authors were influenced by three factors: (1) different bacterial strains; (2) different races of bacteriophage; (3) composition of the medium.

Our ordinary peptone broth is set at P_H 6.6; complete lysis occurs with all our races of bacteriophage at this P_H. We reported in a previous paper[8] that

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our phage do not weaken at P_H 4.8. At P_H 4.4 the activity is decreased about 50 per cent or more and at P_H 4.0 the phage are completely inactive and do not recover any more.

D'Herelle's suggestion to use glucose broth for isolation of bacteria from bacteriophage would be very useful and simple, if two conditions were fulfilled: (1) the acidified medium should not inhibit bacterial growth; (2) this acid medium should completely destroy phage. We thought it worth while to make a detailed analysis of this method for isolation of bacteria. Several factors should be considered: (1) the composition, the sugar content, and the reaction of the medium; (2) the intensity of bacterial growth and the development of resistant bacteria; (3) the influence of the H-ion concentration on the reaction of phage and the relation of phage to resistant bacteria; (4) the use of trypsin for detection of phage inside of bacterial bodies; (5) the influence of a prolonged incubation on the behavior of bacteria and phage; (6) a reliable method for reading and interpretation of the obtained results.

EXPERIMENTS

I. In order to determine the influence of the sugar concentration on the reaction of the medium, the bacterial growth, and the lytic principle, the following experiment was carried out: To a series of tubes containing 10 c.c. of broth, dextrose was added in various concentrations (0.01 per cent, 0.1 per cent, 1 per cent and 5 per cent). The reaction of all the tubes was set at P_H 7.0. To each tube Shiga phage were added in a sufficiently large quantity to cause complete lysis (1 c.c.). Each tube was then seeded with a loop of Shiga bacilli. A second series of tubes with the same sugar content, as the first series, did not contain bacteriophage. As controls served: (1) a tube with plain broth and the above amount of phage; (2) a tube with plain broth and bacteria; (3) a tube with plain broth, bacteria and phage. All the tubes were kept for twenty-four hours at 37° C. The tubes of the first series were perfectly clear with few exceptions; similarly the first and third controls. The tubes of the second series were more cloudy than the second control. Transfers were made on plates from the tubes of the first series and five drops of phage were added to each plate. Transfers of the cloudy tubes of the first series showed resistant bacteria on plates. The contents of the phage tubes were pulled through the Berkefeld filter and tested on plates for the presence of phage. Filtrates of every tube of the first series showed phage on plates. After the incubation, the H-ion concentration of all the tubes was determined. Control No. 1 did not show any changes, controls Nos. 2 and 3 showed only slight ones. Tubes seeded with bacteria only, showed a gradual increase of acidity corresponding to the sugar content. The highest acidity obtained was P_H 4.7, which could be observed in tubes with 1 per cent glucose; tubes with a higher glucose content than 1 per cent did not show a higher acidity. Tubes with bacteria and phage, showed much less acidification of the medium. In tubes with phage and a large number of resistant bacteria, the H-ion concentration was almost the same as in the corresponding tubes without phage. In all tubes which contained bacteriophage before seeding with bacteria, the lytic principle could be found in the filtrates after the incubation. A complete destruction of phage was not found in a single tube. This experiment

was repeated by using asparagin medium instead of broth, the arrangement being otherwise the same. The results were identical with those obtained with broth.

II. A great majority of bacteriologists still use D'Herelle's method for the interpretation of their results with bacteriophage. To avoid objections that the above experiment was carried out with large quantities of phage, the following experiment was made. Ten tubes were filled with 10 c.c. of 1 per cent dextrose broth. This sugar concentration was selected, due to the fact that this amount was sufficient to produce high acidity of the medium, while the bacterial growth within twenty-four hours was quite heavy. In addition, the first tube obtained 1 c.c. Shiga phage, the second tube obtained 0.1 c.c. phage, the third 0.01 c.c. phage, etc. A duplicate series with plain broth was prepared in the same manner. One tube with dextrose broth and bacteria, and another tube with plain broth and bacteria served as controls. All tubes were seeded with one loop of Shiga bacilli. The lytic power of the phage tubes was tested before seeding with bacteria. After twenty-four hours incubation at 37° C. the filtrates of these tubes were again tested for the presence of phage. Usually the control tube with dextrose showed a heavier bacterial growth than the tube with plain broth. A partial or complete disappearance of phage was observed in tubes containing dextrose and smallest amounts of phage, while the corresponding tubes in plain broth showed phage in small quantities. D'Herelle and many others have repeatedly observed that small amounts of phage favor the development of resistant bacteria. After twenty-four hours incubation, the bacteria in all tubes were tested on plates against the phage. It was found that the tubes where the phage disappeared always contained resistant bacteria. We have observed very often, that sugar-containing media show a heavier bacterial growth than those without sugar. This observation can easily be explained, due to the fact that the addition of a suitable sugar to the medium increases its nutritive power. Such increased bacterial growth causes a disproportion between phage and bacteria, and leads to the formation of resistant bacteria. This second experiment seemingly contradicts the first. The results of this experiment indicate that the phage are destroyed only in comparatively small quantities.

Such a conclusion would be perfectly in accord with D'Herelle's conception about the relation between resistant bacteria and phage; he assumes that resistant bacteria destroy phage.

III. Da Costa Cruz[9] and some others found that the phage enter the dead bodies of the susceptible bacteria. This fact induced us to consider the possibility that the phage enter the bodies of resistant bacteria and remain there inactive. Such an occurrence in a culture containing phage and a large number of resistant bacteria would distinctly decrease the amount of the phage. If the quantity of phage were large, the decrease could be easily overlooked; if the quantity were small, it is possible that all the phage enter the bodies of the resistant bacteria and the filtrate of the culture will not contain any phage, when tested on plates. It is generally accepted as a fact that the multiplication of phage occurs at the expense of the susceptible bacteria. Bacteriophage which enter the resistant bacteria would not be able to multiply.

In order to determine whether the phage are destroyed by resistant bac-

teria or enter these bacteria and remain there inactive, but alive, the following experiment was carried out: We prepared 10 tubes, each with 20 c.c. of 1 per cent dextrose broth, decreasing amounts of phage (2 c.c., 0.2 c.c., etc.), and one loop of Shiga bacilli. Three drops were removed from each tube and tested on plates. The tubes and plates were then incubated for twenty-four hours at 37° C. After the incubation, the tubes were heated for one hour at 55° C. and the reaction adjusted to P_H 7.5. Fifteen c.c. of the contents of the tubes were pulled through the Berkefeld filter and tested on plates for the presence of the lytic principle. To the filtrates and remaining contents of the cultures, trypsin was added q. s. 1 per cent. The trypsin was previously tested for the presence of phage, and the results were negative. The trypsin-containing tubes were then incubated for forty-eight hours at 37° C., pulled through the Berkefeld filter and tested on plates. The results showed that the filtrates of cultures exert the same lytic activity before and after tryptic digestion. The filtrates of digested cultures show a stronger phagic activity than the corresponding digested and undigested filtrates; this difference increases toward the lower dilutions. In some low dilutions, the filtrates of cultures before and after tryptic digestion are perfectly negative, while the corresponding filtrates of digested cultures show a more or less distinct phagic activity. This increase of phagic activity in the filtrates of digested cultures can be explained only by the fact that the phage are liberated by tryptic digestion from the bacterial bodies. The tubes with negative filtrates contained a large number of resistant bacteria; the degree of resistance was tested on agar-plates by adding from cultures one loop of bacteria to increasing amounts of phage. The absence of phage in plain filtrates and the presence in filtrates of digested cultures with resistant bacteria indicate that the phage are in this instance liberated from the bodies of the resistant bacteria. These findings justify the following conclusions: (1) resistant bacteria do not destroy phage; (2) bacteriophage which enter the bodies of resistant bacteria can be liberated by tryptic digestion; (3) phage liberated from resistant bacteria are not derived from trypsin and possess the property of regeneration as any other phage; (4) filtrates of cultures with a large number of resistant bacteria and a small amount of phage do not contain any phage; (5) the same cultures digested again show phage; (6) trypsinized culture filtrates cannot contain phage, if they did not contain it previous to the digestion. D'Herelle's classification of bacteriolysis in two acts is therefore justified. The first act occurs regardless of whether the bacteria are susceptible or resistant, but belong to the same susceptible strain; in the second act, the actual lysis occurs only with susceptible bacteria.

IV. These findings induced us to consider that the bacteriophage may possibly enter the bodies of any bacteria and there produce lysis if the bacteria are susceptible, and remain inactive, but alive, if the bacteria are resistant. To test these possibilities, the experiment was worked out in a manner similar to the foregoing. Instead of the resistant bacteria of the susceptible strain, Bacillus subtilis and Bacillus pyocyaneus were employed. The results were entirely negative. There was no difference between the filtrates of cultures before and after the incubation and between the filtrates of cultures before and after tryptic digestion. These experiments were repeated several times; the results were always negative.

V. In the following experiment the influence of time was studied more closely. It was possible that a twenty-four-hour incubation was not sufficient to test completely the phage for the resistant bacteria. To ten flasks with 90 c.c. of 1 per cent dextrose broth, phage was added in decreasing amounts. To the first flask, 10 c.c. of Shiga phage were added and mixed with the broth; 10 c.c. from the first flask were transferred to the second flask and mixed with the broth, each succeeding flask thereby containing ten times less phage. In this manner the same concentrations of phage were prepared as described in previous experiments. Before seeding with bacteria, the phage dilutions were tested on plates. All flasks were seeded with 1 c.c. of a twenty-four hour old culture of Shiga bacilli. After a twenty-four-hour incubation, 10 c.c. were removed from each flask, filtered through the Berkefeld filter, and tested on plates. Ten c.c. from each flask were subjected to a forty-eight hour tryptic digestion, pulled through the Berkefeld filter, and tested on plates. This procedure was repeated on the fourth day, and after one and two weeks. The filtrates of the cultures were also digested by trypsin. The H-ion concentration of every flask was at the same time determined. The obtained results show that the highest degree of acidity in a 1 per cent dextrose broth is obtained within twenty-four hours, and that a longer incubation does not produce any further changes. Similar were the changes in the lytic activity of phage. Bacteriophage, which disappeared after twenty-four hours, could be liberated after two weeks as easily as after a twenty-four-hour incubation. A prolonged incubation in a sugar medium has therefore no influence on the purification of bacteria.

Sugars which are not decomposed by the bacterial ferments have no influence on the development of resistant bacteria; the results are the same as in plain broth. These observations were made previously by D'Herelle and some other authors.

VI. We found it worth while to determine what changes would occur on the phage if the initial reaction of the sugar medium would be higher than P_H 7.0. Six series of phage dilutions were prepared: one with plain broth, the other with 1 per cent dextrose broth at P_H 7.0. In the same manner phage dilutions were prepared at P_H 8.0 and P_H 9.0. All these tubes were seeded with typhoid bacilli and kept for twenty-four hours at 37° C. The H-ion concentration and the phage content were determined before and after the incubation. At P_H 8.0 and P_H 9.0 there was no difference between phage dilutions in plain broth and in dextrose broth. The bacteriolysis was quite regular. The differences between the sugar-free and sugar-containing phage dilutions at P_H 7.0 were the same, as already described. The findings indicate that these changes in the behavior of bacteria and phage occur only in a medium slightly acidified by a fermentable sugar.

VII. The filtrates of cultures were used in the above experiments for the interpretation of phagic activity. Simple transfers from cultures to agar plates were employed only for the detection of resistant bacteria. Very often such transfers give regular growth on plates, while the filtrates of the same cultures show strong phagic activity. Many contradictory findings can easily be explained by the fact that cultures were tested on plates instead of their filtrates. The following experiment gives an illustration of the great differences in results, if

cultures were used instead of filtrates. Ten tubes, each containing 10 c.c. of broth and decreasing amounts of typhoid phage were seeded with one loop of typhoid bacilli. These tubes were then kept for twenty-four hours at 37° C. After the incubation, one loop from each tube was transferred to plates. The contents of the tubes were then pulled through the Berkefeld filter and tested on plates. One loop of the filtrate and one loop of the original typhoid culture, used in this experiment, were spread on agar plates. It is obvious that to the plates of the second series more bacteria were added than to those of the first series. We could therefore expect less phagic activity on the plates of the second series. The results were contrary to our expectations and can be explained only as due to the appearance of resistant bacteria in the broth cultures. This explanation was verified in the following way: One loop from each culture was transferred to a plate and five drops of phage were added; two drops of the phage were sufficient to produce a perfectly sterile plate. Several plates showed a regular growth, while the corresponding plates from the filtrates showed a strong phagic activity. The results obtained indicate that the transfers of cultures on plates are not useful for the determination of the presence of phage; the results are more accurate if the filtrates of cultures are used instead of simple transfers; the filtrates represent the free phage in the culture fluid at the time of filtration.

DISCUSSION

Our experiments could not confirm the findings of Asheshov and Seiser, that the presence of glucose in the medium hastens the bacteriolysis. Some of our observations agree with those of D'Herelle: (1) nonfermentable sugars have no influence on the bacteriolytic process; (2) the bacteriolysis is not appreciably affected if phage in large quantities are present. Our findings disagree with those of D'Herelle concerning small amounts of phage. According to D'Herelle the acidification of the medium occurs before the regeneration of phage takes place. In almost all instances we observed regeneration of phage, even if incomplete; the regeneration was very often disturbed by the appearance of resistant bacteria. This fact could be noticed especially when the reaction of the medium changed slightly acid due to the fermentation of sugar. If the acid, formed by the decomposition of the sugar, was not sufficient to change the reaction of the medium, the regeneration of phage was regular. The produced acidity in the sugar medium was never higher than P_H 4.7, and this degree of acidity was not sufficient to destroy the phage completely. Very small quantities of phage were not found in the filtrates of cultures after the incubation but were recovered by tryptic digestion of the culture. Our findings concerning liberation of phage from resistant bacteria by tryptic digestion are contrary to those of D'Herelle. He claims that the phage do not enter the bodies of resistant bacteria but are destroyed by them. By using the same method we could not detect any bacteriophage in the bodies of nonspecific bacterial strains. It is generally accepted that the optimal activity of phage takes place within a certain range, even if they do not agree concerning the exact limits. We found that there is a decreased activity of phage below this range, the lower limit being P_H 4.8. Within this last range the above described phage phenomena

occur in a sugar medium; the bacteria still show a comparatively good growth, while the phage are less active. This disproportion leads to the formation of resistant bacteria. A number of bacteriophage enter the bodies of resistant bacteria and remain there inactive. These bacteriophage do not pass through the Berkefeld filter, and if there are no free phage left in the culture, the Berkefeld filtrate will not contain any phage. The Berkefeld filtrate represents the free phage in a culture. The filtrate of a trypsinized culture contains the free phage and those liberated from the bacterial bodies.

SUMMARY AND CONCLUSIONS

1. A fermentable sugar favors bacterial growth but not bacteriolysis.

2. A nonfermentable sugar has no effect on bacterial growth or on bacteriolysis.

3. Media, acidified by sugar fermentation, have no visible effect on large quantities of phage.

4. Media, acidified by sugar fermentation, favor the development of resistant bacteria, if small quantities of phage are added.

5. Media, acidified by sugar fermentation, do not destroy bacteriophage, regardless of the concentration of sugar or length of incubation.

6. If the sugar fermentation does not affect the reaction of the medium, the bacteriophage behave the same as in a sugar-free medium.

7. Bacteriophage enter the bodies of resistant bacteria of a susceptible strain but remain there inactive.

8. Bacteriophage inside of bacterial bodies can be liberated by a forty-eight hour tryptic digestion.

9. Bacteriophage do not enter the bodies of nonspecific bacteria.

10. Filtrates of cultures should be tested on plates for interpretation of lytic activity. Simple transfers of cultures lead to erroneous conclusions on account of the appearance of resistant bacteria.

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FAILURE OF THE MOUSE TEST TO DEMONSTRATE THE PRESENCE OF TYPE I PNEUMOCOCCUS IN SPUTUM. AN UNUSUAL INSTANCE*

By Ruth Gilbert, M.D., and C. K. Davenport, A.B., Albany, N. Y.

SO MUCH confidence has been placed in the mouse method for the determination of the type of pneumococcus present in sputum, that an instance in which this procedure failed to demonstrate the presence of Type I in a specimen containing both Types I and III may be of interest.

The patient, a boy of fifteen years, complained of illness on June 12. Definite symptoms of lobar pneumonia did not develop until the fourteenth. The specimen of sputum received on this date was insufficient for a Krumwiede precipitation test; Avery's culture medium and a mouse were, therefore, inoculated.

A few hours after the first specimen was received, a second specimen, large enough to permit the performance of the Krumwiede precipitation test, was submitted. The sputum coagulated readily, and the saline extract made from it was clear. No precipitation, however, was obtained with any of the type pneumococcus sera. This result was confirmed by repetition of the test.

After approximately seven hours' incubation, there was sufficient growth in the Avery medium inoculated from the first specimen to warrant the performance of a precipitation test. With the aid of an agglutinoscope a very faint reaction could be detected at the end of half an hour in the tubes containing Type I serum, both diluted and undiluted. At the end of one and one-half hours the precipitate in these tubes was just visible to the unaided eye. No reaction was obtained in the tests with Types II and III sera. The tests were all made in duplicate.

The physician in charge of the case, on receiving the results of these tests, decided to administer serum, but as the patient had in the past received large doses of diphtheria antitoxin, it was thought necessary to insure adequate desensitization before the large dose of antipneumococcus serum was given. The desensitization was considered complete at eight o'clock, the following morning (June 15). At this time the boy's temperature was 105°, the pulse 110, and respiration 40. Fifty cubic centimeters of antipneumococcus serum were administered. At 12 M. the temperature had reached 105.6°. At 4 P.M. the temperature was 104°. At 8 A.M. an additional 95 c.c. of antipneumococcus serum were administered. At midnight the temperature was 103.5°. At 4 A.M. (June 16) it had dropped to 101.4° and continued to fall until midnight, when it had reached the normal temperature. From then on, the pa-

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tient's recovery was rapid and uneventful, aside from a rash which developed on the nineteenth and gave him considerable discomfort for several hours.

On examination of the blood agar plates inoculated from the sputum and also from the Avery cultures, the predominating colonies were found to resemble those of pneumococci or green-producing streptococci. Suspensions made from the growth washed from these plates were agglutinated definitely by Type I pneumococcus serum, both diluted and undiluted and, much to our surprise, by undiluted Type III pneumococcus serum also. No colonies characteristic of Type III pneumococcus were observed on any of the plates. Of ten colonies fished from the plates before the growth was removed for the agglutination tests, four proved to be cultures of pneumococcus Type I and six of *Streptococcus viridans.*

The mouse that had been inoculated with this sputum was found dead at the end of forty-eight hours. Agglutination, precipitation, and cultural tests demonstrated the presence only of Type III pneumococcus. These results seemed so unusual that a third specimen of sputum was requested on the sixteenth. A mouse inoculated with this specimen was found dead at the end of forty-eight hours. Agglutination and precipitation tests made with the peritoneal fluid showed marked reactions only with the Type III antipneumococcus serum. Colonies of both Types I and III, however, developed on the plates inoculated from the heart's blood and peritoneal fluid.

It was thought possible that Type I pneumococcus from this case might not be pathogenic for mice. Five mice were therefore inoculated with 1.0 c.c. of a twenty-four-hour broth culture. Two died within twenty-four hours, one after two days, and two within four days. The presence of Type I pneumococcus was demonstrated in all five animals. The delayed death of some of the mice inoculated with these large amounts of culture would indicate that the virulence of the cultures for these animals was low.

Although this report covers an isolated case only, it seemed worth recording since the patient appeared to have been so definitely benefited by the serum treatment and the presence of Type I pneumococcus in his sputum would not have been demonstrated by the mouse method alone.

A STUDY OF THE MICRO-KAHN TEST IN SYPHILIS: A REPORT OF 2100 REACTIONS*

By Robert A. Kilduffe, A.M., M.D.,† and W. W. Hersohn
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THE progress of any development in the history of medicine is by evolution rather than revelation, of which circumstance the gradual unfolding of the true relation of the complement-fixation test to the study of syphilis furnishes a striking example.

While the laborious and cooperative studies of a host of workers have culminated in the recognition of the complement-fixation test as the most delicate and constant *single* symptom of syphilis, the coincident appreciation of the inherent complexities of this test, which render it safe and reliable only in the hands of competent and well-trained serologic workers, has led to efforts either to simplify its technic or to find some equally reliable but technically simpler procedure.

To this end the application of flocculation or precipitation tests to the serologic study of syphilis has occupied the attention of numerous investigators but not until the work of Kahn,[1, 2] who demonstrated the essential necessity of extreme care in the concentration of the reagents (serum and antigen), as affecting, not only the occurrence but the constancy of such phenomena in syphilis, have these procedures assumed a position of practical importance.

It is unnecessary, for the purpose in view, to discuss the underlying principles of the Kahn test or to review in detail, or even in brief, the literature which has accumulated bearing upon its value. At present this discussion is concerned, apparently, not with the clinical and laboratory utility or nonutility of the Kahn test in syphilis, but with whether or not the Kahn test should supplant all other procedures and serve as the sole and exclusive serologic procedure in the study of syphilis, concerning which we shall have something to say later.

As has been consistently, and at times rather clamorously proclaimed, a salient feature of the Kahn test is its relative technical simplicity; and in the interests of technical simplicity various workers have endeavored to simplify still further the technic proposed by Kahn and to shorten the time and labor required for his test.

One of the most promising of the methods devised for this purpose is that of Kline and Young[3] which has been reported upon by Kline, Littman and Mill[4] who present a study of 2800 tests.

The purpose of this communication is to report a study of this procedure

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Read before the Sixth Annual Convention of the American Society of Clinical Pathologists, May 13, 1927, Washington, D. C.

in a series of 2116 tests in comparison with the Wassermann reaction upon the same sera.

The comparison was undertaken at the suggestion of Dr. Kline who not only supplied the necessary apparatus, but very kindly demonstrated the technic to us.

The sera examined comprised 1464 secured from the various departments of the Atlantic City Hospital and including sera sent for examination from patients outside the hospital, and 652 from the Genitourinary Clinic of the Municipal Hospital for Contagious Diseases.

For these latter specimens, and also for accompanying data, we are indebted to the Chief of Clinic Dr. C. H. de T. Shivers and his Associate Dr. C. L. Bossert, and their assistants.

The complement-fixation tests were all conducted—as is the routine practice in these laboratories—by the quantitative method described by Kolmer[5] which is now too well known and too widely used to necessitate a repetition of its technic.

Because of the fact that during the demonstration of the micro-test we learned that certain modifications had been made in the procedure as originally described—notably the discarding of the humidor—the technic of the slide test is presented below together with certain details which we have found to be of interest and value.

TECHNIC OF THE SLIDE PRECIPITATION TEST

While ordinary microscopic slides suffice for the performance of four tests, it is more convenient and conserving of time and labor to use glass squares, 3 inches square, and of the thickness of an ordinary microscopic slide, thus permitting 12 to 16 mounts at one time. Before use these glass plates are covered with bon ami paste which is allowed to dry, when it is completely removed by polishing with a soft cloth.

Immediately after use, the paraffin rings are washed off by holding the slides under running scalding water. The slides are then covered with bon ami paste overnight and then cleaned, the paraffin rings not being made until immediately prior to the test.

An instrument is required for making the paraffin rings in which the tests are made on the slide. The one used by us was furnished by Dr. Kline. Its preparation is thus described by Kline and Young:[3]

A piece of No. 28 soft iron wire 14 cm. in length is twice wound tightly about a test tube 12.5 to 13 cm. in diameter forming a double loop and leaving a double shaft about an inch in length. The two shafts are then twisted together to within a quarter of an inch from the free end.

After removing the looped wire from the test tube, a piece of No. 12 cotton thread about a yard long is started from the free end of the shaft after being fastened there by a single twist of the two free ends. Three long turns are made reaching the loop which is then tightly wound with the thread; the winding being continued up to the free end of the shaft where it is fastened between the two ends of the wire by twisting them. The loop is then bent at right angles to the shaft and reshaped by working it against the

end of the test tube and the shaft is then fastened into a wooden holder such as a teasing needle.

The paraffin rings are made by dipping the loop into smoking paraffin (about 120°). We have found that it is an advantage, after removing the loop from the paraffin, to touch it to the surface of an extra slide thus removing any excess of paraffin, and then rapidly press it lightly like a stamp on the slide to be used, thus making neat, regular rings of paraffin of approximately the same thickness and having a diameter of 11 to 12 mm.

Pipettes.—The pipette for delivering the serum is the usual 1 c.c. serologic type graduated in 0.01 c.c.

The pipette for delivering the antigen is a capillary pipette made from glass tubing about 6 mm. in diameter; the delivery end being drawn to such a size that one drop of antigen dilution equals 0.015 c.c. The one used in this series was supplied by Dr. Kline but they may be easily made.

The vials used for preparing the antigen dilution are those recommended by Kahn and were supplied for our use by Dr. Kline.

The Antigen.—The antigen for the test is that described by Kahn.[2] Those used in this series were originally supplied by Kahn and by Kline and later supplies were prepared by ourselves.

After the antigen is finished it is placed in the ice chest for a day or two to precipitate the excess cholesterol and then filtered and kept at room temperature. With this precaution it will remain crystal clear.

The antigen titration is an important preliminary. A series of dilutions of antigen and normal saline are made in the following proportions: 1:1; 1:1.1; 1:1.2; 1:1.3, etc., these mixtures being made as described below, *using not less than 1 c.c. of antigen to start.*

In one of the vials 1 c.c. of antigen is placed and 1 c.c. of normal saline in the other vial. The salt solution is poured into the antigen and, *without waiting to drain the salt solution vial,* the mixture is poured back and forth five or six times. This mixing should be rapidly done. The result is a somewhat cloudy, opalescent solution which should be used at once.

A similar procedure is performed with a mixture of 1 c.c. of antigen and 1.1 c.c. of normal saline and so on.

These various antigen dilutions are now tested by the technic described below with a series of negative and positive sera to find the antigen dilution giving clear negatives with negative sera and well-marked positives with positive sera and the dilution thus found satisfactory is increased by 0.1 c.c. In other words, if the 1:1.1 dilution is satisfactory, 1 c.c. of antigen is diluted with 1.2 c.c. of normal saline for the test. This dilution is then prepared and tested with a series of known negative and positive sera to check the titer.

The Serum.—These are obtained as for the Wassermann test, *care being taken that they do not contain blood cells or foreign particulate matter.* Before use they are inactivated at 56° C. Kline and Young recommend an inactivation period of thirty minutes. Our routine is inactivation for fifteen minutes which was adhered to in this series.

The Test.—The improvised humidor suggested in the original paper of Kline and Young has been found not to be necessary if the tests are performed

in a warm, humid atmosphere without drafts, the slides, pipettes, etc., not being chilled. The slides are placed upon a mat of heavy filter paper (one-fourth to one-half inch thick) or upon a piece of felt. If necessary the surrounding temperature may be raised by several Bunsen flames. *Not more than 12 to 16 tests are done at one time.*

If more than forty-five minutes are to be used in the performance of the test, it is better to make a new antigen dilution as these dilutions are not suitable for use over prolonged periods. A preliminary test of the antigen with several negative and positive sera is advisable.

With everything in readiness, 0.05 c.c. of serum (still warm from inactivation) is pipetted into one of the paraffin rings, twelve to sixteen sera being pipetted in series, using a fresh pipette for each serum.

With the capillary pipette one drop (equivalent to 0.015 c.c.) of antigen dilution is then added. If overdosage occurs through error in pipetting, the mixture overflows the paraffin ring, thus indicating the error.

After the addition of the antigen to all the sera (a matter of a second or two) the antigen and serum are *thoroughly* mixed by the flat end of a tooth pick, a fresh one being used for each serum. Following this mixture, which is rapidly done, the slide is held by the edges between the fingers and rocked with a rotary motion for two minutes *by the watch.* If the paraffin rings have been properly made this agitation may be quite vigorous without spilling the serum-antigen mixture.

When the two-minute period is over the slide is placed upon the microscope stage and readings are made immediately with the low power of the microscope, using reduced light as in studying urine sediments and the coarse adjustment to penetrate all the levels of the field.

Readings.—A negative reaction shows a clear, homogenous field without precipitate.

Positive reactions are indicated by the appearance of precipitates, recorded as plus-minus, plus one, plus two, plus three, or plus four in accordance with the size of the particles and their number.

In doubtful cases or in case of technical mishaps, the test may be repeated and it is, perhaps, advisable that it be done routinely in duplicate *using different antigens.*

Kline and Young in accord with Kahn, regard plus-minus and plus one reactions as without diagnostic significance in which, in the interests of the patient, we concur although, as will be shown later, these are not always without some significance in cases of syphilis under treatment.

It is obvious that this technic is the acme of simplicity and exceedingly conserving of time. Nevertheless, it requires exceeding care in its minutiae to prevent the occurrence of false, misleading, or confusing reactions. Sources of error may be listed as follows:

1. Performance of the test at too cool a temperature.
2. Chilled or cooled apparatus or sera.
3. Use of sera containing foreign particulate particles.
4. Dirty glassware.

5. Improperly prepared or titrated antigen.

6. Improper preparation of antigen dilutions.

7. Neglect of thorough mixture and agitation of serum and antigen mixture.

8. Delay in preparing or reading the tests (leading to disturbance of quantitative proportions by evaporation).

9. Personal equation in reading the results, *especially as regards the weakly reacting sera.*

10. Errors of technic.

The advantages of the micro-Kahn test as compared to the three tube Kahn test are that it requires less apparatus (no test tubes, rack, shaking machine, etc); less time and labor; less serum (one-seventh the amount is required for the micro-Kahn test); and the fact that the results are often more easily read.

All the micro-Kahn tests were made, read, and recorded before the Wassermann tests were completed.

RESULTS OF AUTHORS' SERIES

In reporting the relative total agreement of the micro-Kahn test with the Kolmer quantitative complement-fixation test it is necessary to decide upon the status of the plus-minus and plus one micro-reaction.

Of the 2116 sera tested 468 or 22 per cent were Wassermann positive. If, according to Kline and Young, the plus-minus and plus one micro-reaction is regarded as without significance and thus in accord with a *negative* Wassermann, there were 299 positive micro-reactions (plus two or over) or 14 per cent positive reactions.

This is a total relative agreement between the two tests of 63 per cent.

If, on the other hand, the plus-minus and plus one micro-reaction is accorded significance and is thus in agreement with a *positive* Wassermann reaction, there were 425 positive micro-Kahn reactions or 20 per cent of the total number of sera or an agreement between the two tests of 90 per cent.

While we are quite willing to grant the difficulty of interpreting the diagnostic significance of the plus-minus micro-reaction, we are satisfied that the plus one reaction cannot be entirely disregarded and warrants a further study of the particular case and, further, that both plus-minus and plus one reactions are of some significance in the case of syphilis under treatment.

Procedures proposed for general adoption in the serologic study of syphilis must be applicable to unfavorable as well as to favorable situations. It is of interest, therefore, to consider the effect of physical characteristics of the serum as affecting their suitability for the micro-Kahn test.

Hemolyzed sera or those with a high icterus index do not interfere with the reaction. Chylous sera, or those contaminated with bacteria, molds, or particulate foreign particles are unsuitable as pseudoreactions resembling a plus-minus or plus one reaction occur.

The micro-Kahn reaction with sera anticomplementary to the complement-fixation test is of interest, the results of 24 such sera being shown in Table I.

TABLE I

REACTIONS WITH ANTICOMPLEMENTARY SERA

NO.	MICRO-KAHN	SOURCE	REMARKS
1.	0	Muni.	Known luetic
2.	+	"	" "
3.	+	"	Luetic }
4.	±	"	" } same case, specimens one week apart
5.	±	"	" }
6.	+++	"	"
7.	+	"	"
8.	++	"	"
9.	0	"	"
10.	±	Disp.	Nonluetic; serum cloudy and contains molds
11.	+	Hosp.	No evidence of lues
12.	0	"	Same case as 1; Wassermann later 44400 and Kahn negative. Luetic
13.	0	"	No evidence of lues
14.	+++	"	Luetic
15.	0	"	Later Wassermann 11100. No clinical evidence of lues
16.	0	"	No evidence of lues
17.	0	"	No evidence of lues
18.	No reading possible	"	Cord blood; hemolyzed
19.	+++	"	Luetic
20.	No reading possible	"	Cord blood; hemolyzed
21.	0	Muni.	Luetic; under treatment two years
22.	0	"	Luetic; primary lesion; no treatment.
23.	0	Hosp.	Luetic; previous Wassermann positive
24.	++++	"	Luetic; later Wassermann positive

Several conclusions are obvious from a study of this table:

a. The micro-Kahn test gives a definite reading with a high percentage of anticomplementary sera; this, however, is not invariably the case.

b. The micro-Kahn test may give a clear-cut false negative reaction in the presence of syphilis.

c. The micro-Kahn test may give a doubtful positive reaction in the absence of syphilis.

d. Anticomplementary reactions, and *especially when these occur repeatedly and with fresh serum*, are followed by a positive Wassermann reaction in a definite number of cases.

This is in accord with the feeling of many serologists that when persistent anticomplementary reactions are encountered which cannot be explained by technical factors, the probability is in favor of syphilis.

Interest also attaches to the results of the micro-Kahn tests upon cord bloods of which there were 97 in this series.

Of these three were positive to both tests: W* – 42000; K† – +; W – 4100; K – ++++; W – 44444; K – ++++.

In all of these cases syphilis was proved. There was, therefore, 100 per cent agreement of positive reactions.

While all of the Wassermann reactions were clear-cut negatives, there were four (hemolyzed) sera with which micro-Kahn readings could not be made.

The statistics thus far apply to the total sera tested. Particular in-

*W = Wassermann test.
†K = Kahn test.

terest attaches, however, to the 652 sera from the clinic because complete clinical data is available.

Of these 652 sera 235 or 36 per cent were Wassermann positive. If all degrees of micro-Kahn reactions are regarded as true positive reactions, there were 208 or 32 per cent positive reactions, an agreement between the two tests of 88 per cent.

If, on the other hand, the plus-minus and plus one reactions are disregarded and classed as corresponding to negative Wassermann reactions then the total number of positive micro-Kahn reactions is 137 or 21 per cent, the agreement between the two tests falling to 58 per cent.

In view of the complete clinical check obtainable on these cases we feel that, if technical errors are eliminated, the weak micro-reactions cannot be dismissed as without significance. While we would decline to attribute diagnostic significance to the plus-minus reaction we believe its occurrence should lead to a repetition of both the Wassermann and the micro-Kahn tests as well as a thorough clinical search for evidence of syphilis, and, in the case of syphilis under treatment we believe the weak reactions can be classed as true positive reactions.

In the 652 sera under consideration there were 40 or 6 per cent in which the results of the two tests disagreed, the Wassermann being negative and the micro-Kahn plus two or more. Of these 40 sera, 36 were from syphilitic and 4 were from definitely nonsyphilitic patients in whom neither clinical nor serologic evidence could be obtained on repeated examinations.

In 30 cases the Wassermann reaction was negative and the micro-Kahn test was plus-minus or plus one. Of these evidence of syphilis was obtained in 24 cases. There were, therefore, 70 sera or 10 per cent in which the micro-Kahn test was positive in varying degree, 80 per cent of these sera being syphilitic.

There were 65 sera or 9 per cent in which the Wassermann reaction was positive and the micro-Kahn test negative; in all of these cases definite evidence of lues was obtained.

There was, therefore, 9 per cent of false negative micro-Kahn tests.

In 41 cases, not included in those noted in the preceding paragraph, the Wassermann reaction was positive and the micro-Kahn test plus-minus or one. All of these cases were syphilitic, the weak-micro-reactions, therefore, having a definite significance, what is of importance being the fact that in these cases giving weak micro-reactions the Wassermann reaction was never indeterminate but aways distinctly positive.

In view of the relative proportion between the sera from the clinic for which clinical data were obtained, and the other sera, (1464) of the series, for which we were not always able to obtain clinical data, we feel that conclusions drawn from the smaller series may be applied without impropriety to the larger series. This is borne out by the statistics of the larger series which—as concerns agreements and disagreements—are quite comparable to those of the smaller.

Thus, 217 or 1464 sera, or 14 per cent, were positive to the micro-Kahn test and 233 or 15 per cent were Wassermann positive, a total relative agree-

ment of 91 per cent. If the weak micro-reactions are counted as negatives, the number of micro-Kahn positive reactions becomes 162 or 11 per cent and the total relative agreement 78 per cent.

We believe the incidence of false negative and false positive reactions proved in the smaller series is applicable also to the larger series in which proof was not always available.

In common with other observers using precipitation tests based upon the principles expounded by Kahn we found a definite number of sera (281 or 13 per cent) in which there was disagreement between the two tests, the Kolmer being positive or the Kahn negative or vice versa.

Thus, there were 79 (39 hospital and 40 clinical sera) in which the Wassermann was negative and the micro-Kahn plus two or more, an incidence of 3 per cent; and 53 (23 hospital and 30 clinic) in which the Kahn was doubtful (plus-minus or one) and the Wassermann negative, an incidence of 2 per cent.

On the other hand there were 125 (60 hospital and 65 clinic) sera in which the Wassermann was positive and the Kahn negative, an incidence of 5 per cent; and 73 (32 hospital and 41 clinic) in which the Wassermann was positive and the Kahn doubtful, an incidence of 3 per cent.

These figures are comparable to those of other workers, and furnish an obvious lesson to those who desire to see it.

The results of the comparison described above are comparable to those reported by Kline, Littman and Mill,[4] in a study of 2800 tests, in their series an agreement of 94.9 per cent, and in our series an agreement of 88 to 90 per cent, being obtained. Kline, Littman, and Mill also report an agreement of 95.9 per cent with the regular three tube Kahn test, a phase of the question not studied by us.

It thus appears that for those who desire to use the precipitation test as a check upon the Wassermann reaction, or as an *additional means* for the serologic study of syphilis, the micro-Kahn test serves as a suitable method.

We desire to discuss also, however, a further aspect of this question of paramount importance, namely, can the precipitation reactions, and especially those devised by Kahn, safely *supplant* the complement-fixation test in the serologic study of syphilis?

The present literature concerned with the Kahn test does not appear to be interested in the determination of the value of this procedure as an additional and further means for the serologic study of syphilis but rather with a persistent agitation for its *exclusive* use for this purpose, a sometimes even caustic demand that all other methods shall at once be discarded in its favor.

If it be objected that the results of our investigation cannot apply to those conducted upon the macroscopic method described by Kahn, we wish to call attention to the following facts as a preliminary to our discussion:

1. The principles advanced by Kahn, namely, the type of antigen, the relative proportions and concentrations of the serum and antigen, and the thorough mixture and agitation of the two are adhered to in the micro-test.

2. Kline, Littman and Mill have shown that there is an agreement of 95.9 per cent between the micro- and macro-Kahn tests.

3. Kahn[6, 7] himself has stated that, providing the principles of thorough mixture and agitation as well as proportionate adjustments of the serum and antigen quantities are adhered to, satisfactory and "highly accurate" micro-reactions can be performed with as little as 0.025 and 0.05 c.c. of serum, even quantitative reactions being possible with these small quantities.

Finally, if these premises are not acceptable, then the remarks to follow may be considered as applying to all precipitation tests based upon the principles formulated by Kahn and as drawn from the personal experience of one of us (R. A. K.) with the regular Kahn macro-test, upon our present experiences with the micro-test, and upon the experiences of others with the Kahn macro-procedure as reported in the current literature.

The question of which some desire, apparently, to make an issue is: Shall the Kahn test be accepted as an *additional* or an *exclusive* method for the study of syphilis?

We believe that this is a matter to be decided only after a consideration of many factors; that it concerns not only the laboratory and the clinician; the serologist, the syphilographer, and the practitioner at large, but not least of all, the *patient!* We believe, further, that no discussion of this subject can neglect or pass over as irrelevant a consideration of certain basic facts of essential importance.

First of all, it must be taken into consideration that reports of serologic investigations are made, not only to serologists and syphilographers, but to physicians at large and none can deny that *it is not the test but its clinical interpretation* which is of paramount importance.

None can deny, also, that in no small proportion of cases the serologic examination comprises the major portion of the examination for evidence of syphilis; that many physicians exercise but little curiosity as to the delicacy and reliability of the method whereby it is performed, or the training, skill, or experience of the serologist by whom it is performed; and that for all too many, a positive reaction—however obtained—suffices as indisputable evidence of syphilis and a negative reaction as evidence of its absence or cure.

These are facts common to the knowledge of every serologist and syphilographer and recognized by many physicians; why else the discussions of the "menace of the weakly positive Wassermann reaction" and the clinical pleas for an infallible serologic test?

Again, it must be appreciated, as has been emphasized so many times by so many writers, that serologic examinations are not tests for syphilis but tests for evidences of *reaction to syphilis;* that their occurrence depends, not upon the fact that the patient has syphilis, but upon the reaction of his tissues to the infection. Hence, where there is but minimal tissue reaction—as in latent or dormant syphilis—there can be no serologic reaction. An infallible serologic test, therefore, is impossible by the nature of the situation—there is no procedure with which false negative reactions cannot be obtained.

Furthermore, neither the complement-fixation nor the precipitation tests are procedures dependent upon the presence of true or biologically immune

bodies; both are reactions dependent upon variations in the physical characteristics of the serum—markedly constant in syphilis, it is true—but physico-colloidal rather than immune in character.

Finally, the skill of the performer may definitely influence the results of the reaction to be reported to and interpreted by the clinician.

The arguments advanced to support the exclusive use of the Kahn test in the diagnosis and study of syphilis are based upon the following major premises:

1. *Simplicity of Technic.*—A perusal of the literature by one not a serologist, would lead to the impression that the technic of the Kahn test was of such surpassing simplicity that its satisfactory accomplishment could be acquired by any one within a very short time.

Granting the small amount of apparatus and the few reagents required, the test, nevertheless, has its own inherent complexities, not the least of which is the *preparation and titration of a satisfactory antigen; the constant accurate adjustment* of the serum and antigen proportions for the test, and, especially, the *proper reading and interpretation of the results.* It is in the border-line case without clear-cut clinical evidence, in which the clinical evidence is least conclusive, that serologic tests should furnish the utmost in the way of evidence; and it is in the border-line case, the weakly reacting case that the Kahn reactions are the most difficult to read and interpret. Upon this point nearly all observers are in entire accord.

We do not believe that the Kahn test is so simple and easy that any one, no matter what their previous training, can forthwith perform it accurately and reliably. The diffusion of such an impression is unwise, unfortunate, untrue, and fraught with peril for the patient.

We grant the inherent complexities of the complement-fixation test and consider that this very fact should furnish an additional safeguard as tending to restrict the performance of this test to those well-grounded and well-trained and competent to undertake its performance with understanding and a consciousness of the responsibility involved.

Furthermore, we believe that it is easier to teach a technician to perform a reliable complement-fixation test *the results of which shall be clear-cut* and capable of being read with a minimum of confusion than to attain a similar objective with the Kahn test—*this being especially true of weakly positive reactions.*

The Kahn test *is* simple in the minimum of reagents and apparatus required but we do not believe that this simplicity extends to its principles, its satisfactory performance, or its accurate interpretation.

The Wassermann technic is *not* simple, it is true, but the trained serologist is apprised of technical errors by disturbance of the controls or, indeed, by the very character of the reactions he obtains, safeguards not applicable to the Kahn test.

2. *Minimal Time and Labor Required.*—An extraordinary emphasis has been laid upon the fact that the time and labor involved in the performance of Kahn tests are much less than required for the performance of the comple-

TABLE II

COMPARISON OF KAHN AND WASSERMANN TESTS IN SYPHILIS UNDER TREATMENT

NO.	PT.	KAHN	WASSER-MANN	KAHN	WASSER-MANN	KAHN	WASSER-MANN	KAHN	WASSER-MANN	KAHN	WASSER-MANN	KAHN	WASSER-MANN	REMARKS
1	B. J.	4	44400	4	44400	0	44400	0	44000	±	44000			Treated
2	W. G.	0	Antic.	0	44000	0	44400	0	44100	0	44400			"
3	W. B.	0	40000	1	43000									"
4	S.	3	Antic.	±	0	3	44400	±	44400					"
5	P.	0	44410	1	44400	±	44440							"
6	Pe.	0	44000	±	44000	2	22200	2	44000					"
7	M. C.	4	44440	0	33330	0	44000							"
8	W. J.	1	0	±	0	0	0	0	0					"
9	H.	0	10000	0	44400	0	44220							"
10	Ha.	4	44444	3	44444	2	44400	4	44444					"
11	E.	±	44444	2	44440	4	44410	0	40000					"
12	Hu.	1	44400	0	44432	0	44100	0	44000					"
13	F.	0	44444	3	44444	2	44444	4	44441					"
14	C.	1	44400	0	44400	4	44440	0	41000					"
15	W.	2	44400	0	44400	0	44000	0	0					"
16	Po.	2	0	2	44000	0	0							"
17	N.	0	Antic.	0	0	0	0							"
18	Y.	0	0	±	44000	0	0							"
19	Ev.	0	0	0	44400	0	40000	2	0					"
20	Wa.	4	44441	4	44430	4	44200	1	44000					"

TABLE II—CONT'D

NO.	PT.	KAHN	WASSER-MANN	KAHN	WASSER-MANN	KAHN	WASSER-MANN	KAHN	WASSER-MANN	KAHN	WASSER-MANN	KAHN	WASSER-MANN	REMARKS
21	Ca.	0	10000	0	0	0	0							Primary lesion
22	R. S.	0	0	0	0	0	41000	1	13200	0	40000			" "
23	A.	4	44440	3	44440									Treated
24	J. S.	1	Antic.	3	44000									"
25	Y. J.	0	40000	0	21000	0	0							"
26	V. B.	3	0	4	44400	4	44440							"
27	H. I.	1	44000	0	41000	0	0	0	0					"
28	J. H.	1	0	1	0									"
29	Wb.	0	40000	0	44400									"
30	A. C.	0	0	0	0									"
31	R. H.	3	44400	4	44400	1	40000	0	0					"
32	B. C.	4	44400	±	40000									"
33	C. U.	2	0	2	44444	3	44444	4	44440					"
34	J. A.	0	44400	0	44400									"
35	R. G.	0	0	0	11000	±	20000	0	20000					"
36	L. B.	1	44400	0	0	0	0							"
37	G. B.	0	44400	0	44400	2	44000	1	44400					"
38	M.	0	0	0	0	4	44400							Primary lesion
39	T.	2	40000	0	0	0	0							Treated
40	R. E.	0	44000	±	Antic.	±	Antic.							"

ment-fixation test and this is advanced as a powerful argument for the exclusive adoption of the Kahn test.

While admitting the fact, we are not inclined to attribute to this argument the paramount importance which has been given to it by others.

We are not convinced of the necessity for precipitate haste in the diagnosis; rather, we agree heartily with whoever said: "Be quick to suspect syphilis but slow to diagnose it." Syphilis, at best, is a refractory disease and, under the most ideal conditions, its treatment is a tedious procedure. So great is the influence of a diagnosis of syphilis upon the future of the patient, so important its relation to his life, his family, his friends, and even his every-day existence that we are anxious to be *sure* rather than precipitate in our diagnosis. We do not regret nor grudge whatever may be necessary in time or labor to render the diagnosis relatively free from error or to safeguard the efficient treatment of this dreaded malady. We are quite confident that with our own serum under examination, time and labor expended in its study are as chaff before the wind and we are willing to grant the patient no less consideration.

"The clinician or internist cannot evade a thorough physical examination because it is time-consuming, nor the surgeon the adoption of an efficient technic simply because it is laborious. The serologist and syphilographer in a similar situation must take the same stand."[8]

3. *Delicacy and Specificity.*—The essential requisite for a satisfactory serologic procedure in syphilis is relative specificity, relative freedom from the occurrence of false positive reactions. The greater the degree to which this attribute can be combined with delicacy, the nearer the approach to the ideal.

Investigations concerning the specificity of the Kahn test are, of necessity, largely based upon a comparison of this procedure with the Wassermann test and the fact, well known to serologists but often not equally as well appreciated by physicians at large, that, unless technical details are described, the phrase "Wassermann test" conveys no idea of the reliability of the method employed, suffices to explain the varying results reported of agreement and disagreement between the Kahn and Wassermann tests.

Granting, however, a delicate, and relatively specific method of complement-fixation, it may be accepted as demonstrated that the Kahn test will agree with the complement-fixation test in from 90 to 95 per cent of cases, the figures depending upon the skill with which both procedures are applied and the extent of the series studied.

It has also been demonstrated, however, that false negative reactions may be obtained with *either* test; with the Wassermann test because of the influence of natural amboceptor in the tested serum, and also of substances whose nature is unknown but which are capable of producing results similar to those caused by an excess of amboceptor,[9, 10, 11, 12] with the Kahn test for reasons not entirely explained; and with *both* tests for reasons inherent in the disease and the reaction to the disease on the part of the patient.

These false negative reactions occur, *not only in dormant, but also in active syphilis and constitute, therefore, a very definite source of error when only one test and only one examination is taken at its face value*—as is the regrettable but too common tendency of many physicians.

Very fortunately, however, such false negative reactions, while occurring with both tests, not infrequently do not occur simultaneously so that one test may be positive and the other negative. We consider this an extremely valuable circumstance as supplying an additional safeguard and *furnishing a potent argument for the coincident use of both procedures.*

Incidentally, while the complement-fixation reaction and the precipitation reaction have much in common in the proffered explanations of their mechanisms, the fact that one may be positive and the other negative on the same serum at the same time suggests a difference, either in the mechanism or the substances involved.

While the occurrence of false negative reactions is of importance in relation to the serodiagnosis of syphilis, it is of still more importance, and especially when malinterpreted, when serologic procedures are utilized as a guide or control of treatment.

Large series of comparative Kahn and Wassermann tests may show a high incidence of agreement and yet the gross figures may fail to bring out facts seen by a comparison of the two tests on the same patient at different times, and under varying circumstances.

We present a series of 40 cases, all syphilities under treatment, upon whom from two to five examinations were made at varying intervals.

We call attention, first, to the constancy of the Kolmer test and its graphic quantitative character; secondly, to the lack of consistent quantitative comparison of the micro-Kahn with this method; and, finally, to the consistent false negative reactions obtained with Cases 2, 3, 9, 12, 16, 18, 19, 22, 24, 29, 34, 35, and 36.

Also note the cases giving doubtful Kahn reactions and clear-cut positive Wassermann reactions and those in which the Kahn test is neither positive earlier nor persists longer than the Wassermann and we do not find, therefore, that the Kahn test is *always*, as has been stated, positive sooner or remains positive longer than the Wassermann in syphilis under treatment.

If these differences are presented in graphic form, as in Chart I, they are even more strikingly apparent.

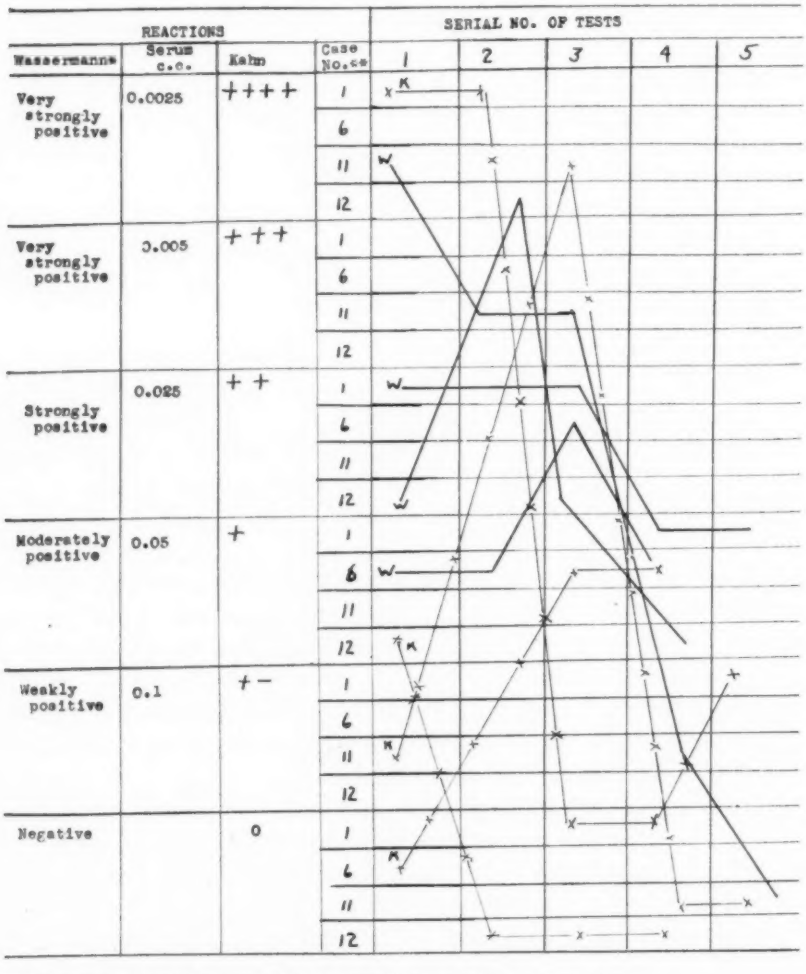
From the numerous comparisons which have been made of the Kahn and Wassermann tests, the latter comprising a variety of methods, including that described by Kolmer which is conceded to be of exceeding delicacy and possessing a high degree of relative specificity, we believe that the Kahn test has been shown to be possessed of a high degree of relative specificity and delicacy. It has also been shown, however, that with either test false negative reactions may be obtained and, what is of still greater importance, the occurrence of false positive reactions has been demonstrated with the Kahn test in a varying number of cases, their incidence, we believe, being between 3 and 5 per cent.

This fact alone constitutes a most potent argument against the *exclusive* use of the Kahn test for the serodiagnosis of syphilis *as long as the acceptance and clinical interpretation of serologic reactions in syphilis is based upon their face value, as is true in no inconsiderable number of cases.*

The next most important single reason against the adoption of the Kahn test as an exclusive method is the fact that, when the Kolmer and Kahn tests (either micro- or macro-methods) are done routinely, variations occur in from 4 to 5 per cent of sera of individuals clinically regarded as syphilitic, and especially of patients under antisyphilitic treatment, these variations consisting of negative reactions with one test while the other is coincidently positive.

CHART I

GRAPHIC CHART OF DIFFERENCE BETWEEN KAHN AND WASSERMANN TESTS IN TREATED SYPHILIS



*Wassermann reaction recorded in smallest reacting serum dose.
**Refers to cases in preceding table.
Wassermann————————
Kahn ————————x————————x————————

4. A great deal of emphasis has been laid on the fact that the Kahn test has been adopted as an exclusive test by the Michigan State Department of Health Laboratories and by the United States Navy.

As previously said in another place:[13] "The fact that the originator of the test is Immunologist of the Michigan State Department of Health Laboratories may be regarded as a factor of some importance in its exclusive adoption by these laboratories. The difficulties involved in the satisfactory performance of Wassermann tests aboard ship or in isolated Naval Stations, and the fact that all Naval Surgeons are not necessarily accomplished serologists" has certainly not been without influence on the adoption of this test by the Navy to the exclusion of the Wassermann. We do not find in these facts any obligation upon the serologic fraternity at large forthwith to do likewise.

We regard as unassailable the assumptions that, in all that has to do with the study of syphilis, the patient is intimately concerned; that, both as to diagnosis and the initiation, continuation, cessation, or resumption of treatment, every possible safeguard should be employed and zealously sought for; that, so great and disastrous and practically impossible ever to eradicate is the stigmatizing aftermath of a false positive diagnosis, and so widespread the calamitous potentialities of a premature cessation of treatment that every possible means, *no matter how laborious or time-consuming,* should be employed to prevent these errors.

We deplore a blind and unquestioning acceptance of any serologic procedure as constituting of itself a thorough, complete, or infallible examination for evidence of syphilis. We deny the suitability of the Kahn or any other precipitation test for this purpose, and we believe that its true value and proper place is as an additional serologic method for the diagnosis and study of syphilis to be used *with and not in place of* the complement-fixation test.

If still another serologic procedure of comparable value to the Kahn and Wassermann tests should come to light, we would rather add it to our armamentarium than discard either.

SUMMARY

A report of 2116 comparative micro-Kahn and Kolmer tests is presented as a result of which it is concluded that the micro-Kahn test is a satisfactory additional method for the serologic study of syphilis.

The suitability of the Kahn test as an exclusive method for the serologic study of syphilis is discussed and the conclusion reached that it should be used *in conjunction with and not in place of the Wassermann test.*

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THE PREPARATION OF POTASSIUM AND SODIUM TETRABISMUTH TARTRATES*

By Philip Adolph Kober,† Chicago, Ill.

INTRODUCTION

THE value of bismuth therapy in syphilis as shown by Cole, Farmer and Miskdjian[1] depends upon the solubility and speed of absorption of the bismuth preparation used. They found that the bismuth tartrates were the most readily absorbed compounds that they studied, but even these compounds required a long time for absorption when suspended in oils.

The following work was undertaken with a view to making a soluble bismuth preparation of definite chemical composition, which could be administered in aqueous solution, free from the slow absorption, nodule formation and danger to fat embolism, due to the use of fat suspensions. For rapid absorption it seemed desirable to avoid the use of the potassium compounds, owing to their objection found by many workers in pharmacology and medicine[2] and therefore most of the work was done on sodium compounds.

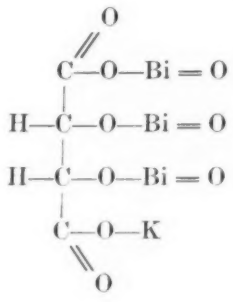
IMPORTANT SOLUBLE BISMUTH COMPOUNDS

Theoretically and actually found, there are many different compounds of bismuth and tartaric acid. Of the water soluble preparations which are probably more effective on account of speedier absorption and greater penetration, all of them also contain in addition to bismuth and tartaric acid, alkali metals, either sodium or potassium or both. Ordinary bismuth salts of tartaric acid have a too great tendency to hydrolyze into insoluble bismuth oxide or basic salts to be desirable for syphilitic treatment. Therefore the more firmly the bismuth is bound to the tartaric acid in the form of a complex, the greater efficacy can be expected as the bismuth must remain in the form of a soluble organic complex long enough to allow absorption from the site of injection and permit distribution throughout the body by means of the blood stream. Chemical tests show that the most firmly bound bismuth compounds heretofore isolated are the so-called tribismuth alkali tartrates,

*Read before the Organic and Biological Group, American Chemical Society, Sectional Meeting, Madison, Wisconsin, May 29, 1926.

†From the Research Laboratories of G. D. Searle & Company.

of which the only known example is potassium tribismuth tartrate. The following formula has been assigned to this compound:



with $4H_2O$

72.8% Bi 67.1% Bi

This compound and its preparation were first described by Rosenheim and Vogelsang[3] in 1906, and has been on the market and used clinically both here and abroad. For reasons stated above, a sodium tribismuth tartrate seemed desirable and possibly of value. No method was available in the literature which described the isolation of this compound. Rosenheim and Vogelsang attempted to isolate the sodium compound but they stated it would not crystallize out similarly to the potassium compound. Klauder[4] makes the statement that the sodium salt is unstable which indicates in the light of the work reported here that the sodium compound had not been successfully isolated. It is probable that Rosenheim and Vogelsang in their experiments, and possibly others too, actually had some of the sodium tribismuth tartrate compound present in their solutions together with an excess of reagents; but in this form its usefulness for syphilitic treatment could not and never did become practical.

ALKALI TETRABISMUTH TARTRATES

On considering the method of Rosenheim and Vogelsang which consisted in digesting bismuth subnitrate with tartaric acid and an excess of alkali, while heating, I came to the conclusion, that from thermodynamic reasoning, the reaction ought to be conducted in the cold or at least, without heating. Furthermore, since the nitrate group does not enter into the composition of the compound desired, its presence may interfere with the reaction. For these reasons the reaction was tried with a slight excess of sodium hydroxide in the cold, using bismuth hydroxide as a source of bismuth, and shaking with a mechanical shaker. The bismuth hydroxide at first dissolved fairly rapidly, but soon it dissolved more and more slowly. A number of experiments, however, showed that the longer I shook the mixture, the more bismuth hydroxide dissolved. At the end of about 144 hours (6 days) the mixture seemed to come to an equilibrium and no more bismuth hydroxide seemed to dissolve. On filtering I had in solution, of course, a sodium bismuth tartrate. A few experiments with small portions of a filtrate soon showed that a half volume of 95 per cent alcohol gave me a copious yield of

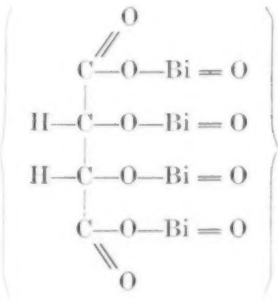
precipitate. The precipitate after washing with 50 per cent alcohol several times to remove the mother liquor and finally with 95 per cent alcohol and drying in the air at room temperature gave a water soluble powder containing 72.9 per cent of bismuth* and 4.6 per cent of water of hydration, or for an anhydrous substance 76.4 per cent of bismuth. Since the anhydrous sodium tribismuth tartrate could not contain more than 74.2 per cent of bismuth, it was evident that this preparation contained more than three molecules of bismuth. The theoretic per cent for bismuth and water for sodium tetrabismuth tartrate is seen in Table I:

TABLE I

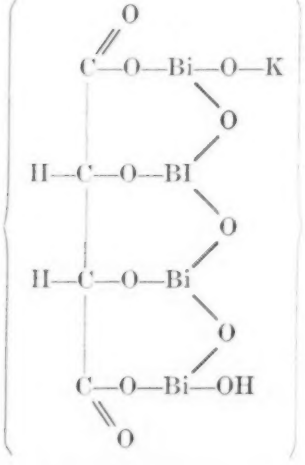
	ANHYDROUS	$1H_2O$		$2H_2O$	$3H_2O$	
	Bi%	H_2O%	Bi%	Bi%	H_2O%	Bi%
Theory for Bismuth Tartrate	74.2	2.09	72.7	71.2	6.01	69.6
Found	76.4				4.60	72.9
Theory for Tetrabismuth Tartrate	77.1	1.63	75.7	74.3	4.73	73.2

From these data I was forced to the conclusion that this sodium tartrate compound was sodium tetrabismuth tartrate and I have tentatively assigned the following formula to it:

Mol. Wt.—1086
77.1% Bi
Formula I



Mol. Wt.—1102
75.8% Bi
Formula II



*The bismuth content was obtained by dissolving 0.500 gm. of the compound in 25 c.c. of water, heating the solution to 50° C. and adding 1.0 gm. of sodium hydrosulphite ($Na_2S_2O_4$) dissolved in 5 c.c. of 10 per cent of ammonia, then filtering, washing, drying and weighing the metallic bismuth.

If these findings and conclusions with the sodium compound were correct, then it ought to be possible to get a potassium compound of tartaric acid with even more bismuth content than any found on the market, since most of them approximate in bismuth content that of a tribismuth tartrate.

On substituting potassium hydroxide for sodium hydroxide in the method developed for the sodium compound, I had no difficulty in getting a fair yield on the first trial using otherwise the exact technic I did for the sodium process. Perhaps by using more suitable proportions of alcohol, since the solubilities of the two substances undoubtedly differ, a greater yield would have been obtained. The analytic data, however, agree with the theory of a tetrabismuth tartrate even more closely than did the sodium compound.

TABLE II

	ANHYDROUS	$1H_2O$		$2H_2O$	
	Bi%	H_2O%	Bi%	H_2O%	Bi%
Theory for Tribismuth Tart.	72.9	2.05	7.14	4.02	70.0
Found.	75.8			2.76	73.8
Theory for Tetrabismuth Tart.	75.8			3.16	73.3

PROPERTIES OF TETRABISMUTH TARTRATES

These tetrabismuth tartrates are finely divided powders, odorless and tasteless, permanent in the air at ordinary room temperatures. At higher temperatures, they seem to suffer a change, probably absorbing carbon dioxide and other acid vapors from the air, so that they require additional alkali before they will dissolve in water. On contact with water, these preparations first form a gel and then dissolve. Accurate solubility determinations have not yet been made; indications are that the solubility is somewhere near 40 per cent; one part of water at room temperature does not dissolve quite one part of these tetrabismuth tartrates, whereas one and a half parts usually will.

Solutions of these tetrabismuth tartrates are alkaline in harmony with the formula, and so far as we know are perfectly stable. The alkalinity can be decreased by titrating with N/10 sulphuric acid, using phenolphthalein as an indicator, to a hydrogen-ion concentration of about 8.4-8.6 at which point the solutions up to 10 per cent are still stable indefinitely. Heated to 70° C. for three-fourths hour the solution seems to be unchanged, while heating for five minutes at 100° produced only a very slight cloud and a slight increase in alkalinity, but which on cooling slowly but practically disappeared.

The alkalinity can be also decreased by absorption of iodine, with the formation of colorless solutions of iodides and iodates of the tetrabismuth tartrates, which also seem stable at room temperatures.

On adding a gram equivalent of acid to solutions of tetrabismuth tartrates a precipitate is produced, which redissolves in an equivalent amount of alkali. This base is very gelatinous and seems to be close to a permanent suspensoid.

These tetrabismuth tartrates are not precipitated by blood proteins, carbohydrates, etc.

TOXICITY

Raiziss, Severac and Windicov[5] quote Sazerac and Levaditi, who were the first to study bismuth tartrates as curative agents for syphilis, as stating that their sodium and potassium bismuth tartrate killed white rats when 5 mg. per kg. was injected intravenously. Raiziss and associates found their potassium tribismuth tartrate, a supposedly tribismuth tartrate, to be tolerated up to 10 mg. per kg., but killing at 15 mg. per kg. Our preliminary results with white rats indicate that these tetrabismuth tartrates are considerably less toxic being tolerated when injected intravenously up to about 25 mg. per kg. Injected intramuscularly these tetrabismuth tartrates are tolerated in something over 600 mg. per kg.

PREPARATION OF TETRABISMUTH TARTRATES

Two hundred grams of bismuth subnitrate were dissolved in 270 c.c. of concentrated nitric acid and made up with water to about 1500 c.c. Then with rapid stirring 300 c.c. of saturated sodium hydroxide (50 per cent) were added to precipitate the bismuth hydroxide in a finely divided condition. The precipitate was then filtered upon a suction filter, washed and resuspended in water, filtered, washed with distilled water three or more times until all of the mother liquor had been removed.

Into a liter bottle or flask were weighed 25.0 gm. of tartaric acid and 75 c.c. of distilled water were added. Then 28 c.c. of saturated sodium hydroxide (50 per cent) were added with stirring and cooling. When the liquid had cooled at 14-15° C. the bismuth hydroxide, prepared as described above, was added and after stoppering the bottle or flask securely, the mixture was shaken on a mechanical shaker for one hour or two, at three hour intervals during the first day. This shaking was repeated two or more times a day, for 6 to 7 days. Longer standing or shaking caused no harm, but the amount of bismuth hydroxide dissolved by the alkaline tartrate solution was hardly increased over the amount dissolved during the first six days. Attempts to shorten the period by heating decreased the amount and purity of the yield.

The mixture at the end of six days was filtered through a porous glass Buchner suction filter, or through a hard filter paper. After washing the undissolved bismuth hydroxide with 50 c.c. of distilled water, the total filtrate equalled 350 c.c.

On adding 175 c.c. of 95 per cent alcohol to the filtrate with stirring, a copious precipitate was obtained which was filtered on a Buchner funnel with suction, washed and suspended in 100 c.c. of 50 per cent alcohol, filtered and washed, resuspended, filtered and washed with 95 per cent alcohol three or four times or until the filtrate was neutral to litmus paper. After drying at room temperatures for several days, the yield was in the neighborhood of 125 gm. or 89 per cent. On substituting the same proportion of potassium hydroxide for sodium hydroxide, the yield was about 30 per cent. Larger yields could undoubtedly be obtained with the potassium compound if larger amounts or other proportions of alcohol were used.

PURIFICATION OF TETRABISMUTH TARTRATES

It seems possible to purify these tetrabismuth tartrates by dissolving in water and reprecipitating with alcohol. The following experiment is of interest in this respect: Two small yields from some preliminary experiments with the sodium compound, one having 13 gm. with a bismuth content of 71.4 per cent and another having 65 gm. with a bismuth content of 67 per cent, were dissolved in 300 c.c. of water together with 5 c.c. saturated sodium

hydroxide. A little excess of alkali is sometimes advisable to prevent the alcohol from precipitating the tetrabismuth tartrate base, through the interaction of the alcohol and the alkali of the complex. On adding 130 c.c. of 95 per cent alcohol with stirring, filtering and washing with alcohol and drying obtained a yield of 59 gm., with a bismuth content of 73.8 per cent.

The fact that mixtures of alkali bismuth tartrates having a bismuth content equivalent to that of a so-called tribismuth tartrate can by reprecipitation give a large yield of a tetrabismuth, indicates if it does not prove that these so-called tribismuth tartrates are mixtures of tetrabismuth and dibismuth tartrates. Preliminary toxicity results show that the tetrabismuth tartrates are much less toxic than these tribismuth tartrates, indicating that the latter contain bismuth or compounds containing bismuth, which easily splits off or ionizes bismuth.

CONCLUSIONS

(1) It is shown that contrary to previous directions, alkaline solutions of tartaric acid dissolved bismuth hydroxide more efficiently at a low temperature. (2) When the reaction is allowed to go to completion, tetrabismuth tartrates result, instead of tribismuth tartrates as has been heretofore assumed. (3) Two new compounds, sodium tetrabismuth tartrate and potassium tetrabismuth tartrate, have been described and the processes given for their preparation. (4) Preliminary results indicate that these new tetrabismuth tartrates are much less toxic than other bismuth tartrates isolated heretofore. (5) That certain data given, indicate, if they do not prove, that the so-called tribismuth tartrates, mostly heretofore used in syphilitic therapy, are only mixtures of tetra- and dibismuth tartrates. (6) Preliminary experiments show that the sodium tetrabismuth tartrate can be put up in an aqueous solution and administered intramuscularly.

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A MONILIA FROM THE RESPIRATORY TRACT*

By Frederick W. Shaw, M.D., Richmond, Va.

AFFECTIONS of the bronchi and lungs due to Monilia Persoon, 1797, are found throughout the tropics, especially in places having a damp climate. The affection may be met with in Europe and in America. Castellani[1] states that in Ceylon the malady is generally due to Monilia tropicalis Castellani, 1910, and that the same fungus may be found in cases coming from South India and from the Malay States. In some cases other species of the fungus may be observed, but it is doubtful whether all of these are really pathogenic.

The genus Monilia Persoon, 1797, is usually defined[1] as Oösporaceae possessing in situ budding forms and mycelial threads, which latter are often long and branched; in cultures mostly budding forms, but sometimes filaments, in which thallospores of the blastospore type are formed. Dextrose and often other carbohydrate media fermented with the production of gas.

Much confusion has resulted from the attempts to classify the genus Monilia, and Castellani has suggested that the classification should be based, not only on morphologic data, but also on biologic characters and immunization phenomena.

The following case was observed in St. Philip Hospital, Richmond, Va.

REPORT OF CASE

G. B., aged twenty-five years; female; married; colored. Admitted January 11, 1926.

Three months previous to admission she experienced stiffness and soreness from the neck spreading downward to the knees. She had toothache for years. Immediately preceding the present attack, there was severe toothache in the right lower jaw. The right jaw was much swollen.

Physical examination: Patient was a well-developed colored woman of about the stated age. Mouth showed a severe pyorrhea. There were no cardiac murmurs. Some dullness over right front of chest extended downward to the fourth rib. Posteriorly, the dullness extended to near the angle of the scapula. Tubular breathing was heard over the upper portion of the right lung, extending downward to the third rib anteriorly, and below the spine of the scapula posteriorly. Occasional râles on coughing were heard throughout the right lung. Râles were occasionally present in the upper left lung. Tubular breathing at left apex. Pectoriloquy over both apices.

Patient did not have the appearance of being acutely ill. Very little sputum and cough; no chills; no sweats. Appetite was fine; she slept well.

X-ray of chest showed an apparently well-advanced tuberculosis involving the upper right lobe. This had progressed to consolidation from the apex downward to the level of the second rib. The condition did not appear healed.

The temperature on admission was 100° F. It varied from 99° F. to 100.2° F. and became normal on February 11, 1926.

The patient received seven drops of a saturated solution of potassium iodide three times a day, beginning on January 28, 1926, and this was continued until her departure from the hospital, February 20, 1926.

*From the Department of Bacteriology, Medical College of Virginia.
Received for publication, February 26, 1927.

THE ORGANISM

Examination of the scanty sputum failed to show the tubercle bacillus, either by microscopic examination or by guinea pig inoculation. The sputum contained small, firm, white granules which when crushed under a cover glass were found to be composed of thickly matted threads and yeast-like bodies.

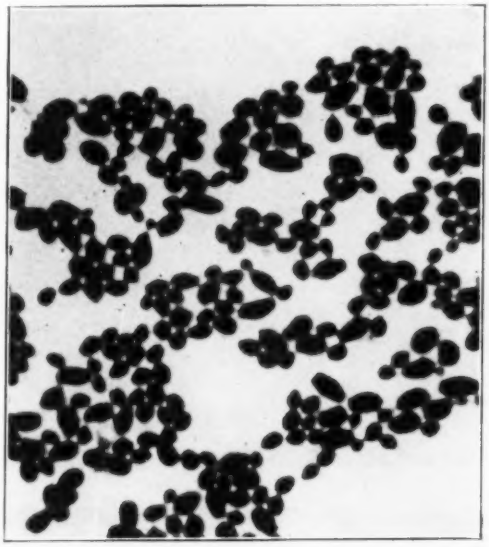

Fig. 1.—Monilia richmondi. Gram stain from growth on dextrose agar.

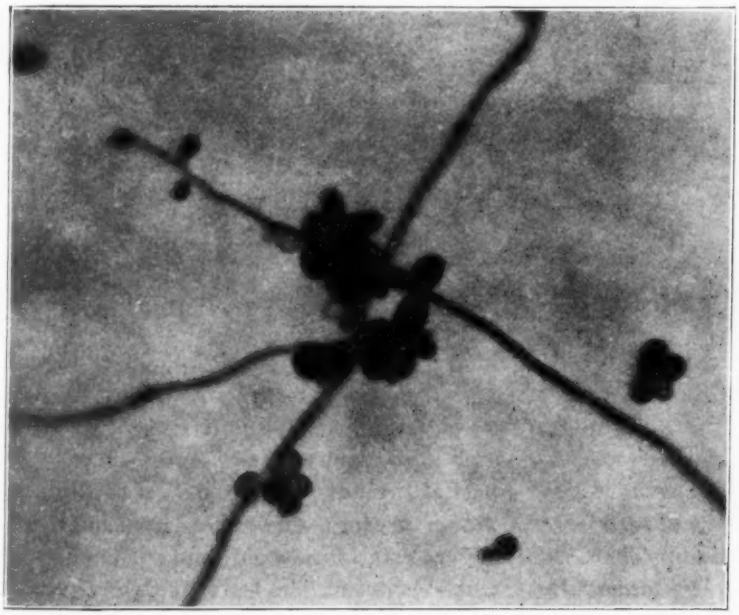

Fig. 2.—Monilia richmondi. Gram stain from growth in dextrose broth.

These granules were planted on maltose agar and the organism isolated. Cultures from the tonsils and teeth were negative for monilia.

The growth on maltose agar which was abundant, was glossy, creamy white with a smooth, raised surface and entire margin. Examination of the growth under the low power of the microscope showed it to be made up of roundish bodies resembling yeast cells. Slide preparations of the growth under the 1.9 mm. objective showed globular yeast-like budding cells, which varied from 1.4 μ to 5.6 μ (Fig. 1).

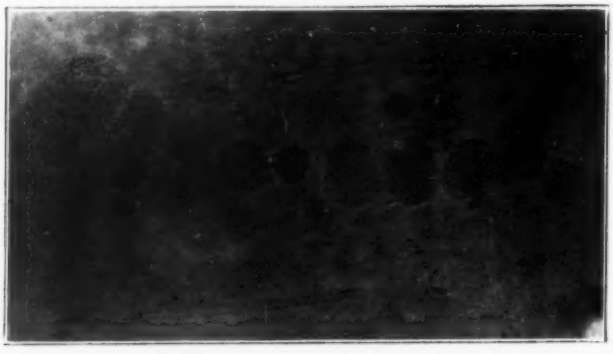

Fig. 3.—Monilia richmondi. Moniliform bodies. Section of growth in gelatin. Unstained.

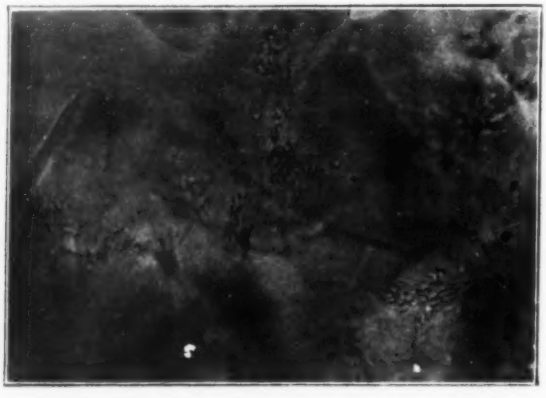

Fig. 4.—Monilia richmondi. Conidiophores and conidia. Section of growth in gelatin. Unstained.

Examination of the growth from dextrose broth showed, in addition to the yeast-like cells, long, septate, branching mycelia (Fig. 2). Spores were seen on the mycelium at the ends and at the junctions of the septa. The mycelium was from 1.4 μ to 1.8 μ in diameter; the segments were from 8.4 μ to 21 μ in length, and the mycelial threads were from 47 μ to 140 μ, or longer.

Milk was rendered alkaline in forty-eight hours without preliminary acidification. There was no coagulation. Acid and gas were formed in dextrose, levulose, maltose, and galactose. The following were not fermented: saccharose, lactose, mannite, dulcite, raffinose, arabinose, adonite, dextrin, sorbite, inulin. No pellicle was formed on broth or Dunham's solution, and

the medium was clear with the growth at the bottom of the tube. Blood-serum was not liquefied.

The organism stained by Gram's method.

No asci were observed.

The growth in a 5 per cent gelatin stab after about a week showed a growth which resembled the root system of a tree. Branched outgrowths occurred from

Fig. 5.—Monilia albicans. Section of growth in gelatin. Unstained.

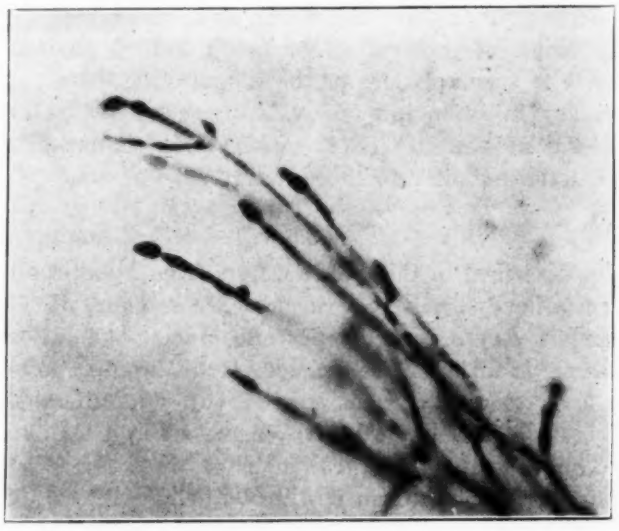

Fig. 6.—Monilia richmondi. Rabbit kidney showing the organism. Stained with dilute fuchsin and decolorized rapidly with alcohol.

the line of stab. These branching outgrowths, when examined with a hand lens, appeared to be made up of small bead-like bodies somewhat akin to the growth of Monilia albicans. When the gelatin culture was hardened with 10 per cent formalin and thin sections were made,[2] the growth was seen to be made up of moniliform bodies (Fig. 3) and structures which resembled conidiophores. The

conidiophores were short and bore conida which were oval in shape and measured 1 μ by 3 μ. Branching of the conidiophores was common (Fig. 4). The production of this type of conidiophore and conidia was not observed in gelatin stabs of Monilia albicans (type species, American Type Culture Collection). The character of the formation of the moniliform bodies in M. albicans is shown in Fig. 5. The articles of the moniliform bodies are always round.

In the preliminary report of this organism[3] it was stated that gelatin was not liquefied. This statement was made as the result of using 10 per cent gelatin. By the use of 3 per cent gelatin and employing the single stab method, or by the use of 5 per cent gelatin and a very heavy inoculation, it was found that liquefaction would begin in about three weeks at room temperature.

ANIMAL EXPERIMENTS

Inoculations into the peritoneal cavities of rabbits or guinea pigs produced no lesions. When injected into the circulation of the rabbit there developed, about the fifth day, tetanoid convulsions of short duration followed by the death of the animal. Necropsy showed the kidneys enlarged and studded with very small, whitish granules; the liver, stomach wall, and omentum contained simliar granules. The organism was recovered in pure culture from a number of the lesions. The guinea pigs received injections of the culture intracardially. Convulsions with paralysis of the hind legs developed on the fifth day; death followed in a few hours. Necropsy showed that the same pathologic changes had occurred in the rabbit.

Stained histologic sections of the rabbit's kidney showed the whitish granules to be due to accumulations of the fungus (Fig. 6).

Intrapulmonary injection into the rabbit produced a caseation and necrosis of the lung at the site of injection; later it produced a general septicemia with lesions similar to those described.

DISCUSSION

The monilia described in this paper differs from Monilia albicans in that it does not form a honey-comb growth on dextrose agar, does not coagulate milk, and presents a very different appearance during the development of the moniliform bodies. It differs more markedly from Monilia psilosis,[4] which in gelatin shows a characteristic pine tree growth with fine hair-like lateral shoots extending from the entire length of the stab. On dextrose agar, M. psilosis produces a rough, yellow growth in contrast to the glossy, creamy-white appearance of the organism here presented.

The specific name of richmondi has been suggested for this monilia.[3]

Thanks are extended to Dr. Thomas P. Haslam, who supplied me with the sputum and the case history.

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THE ORIGIN AND NATURE OF THE WASSERMANN ANTIGEN

By L. G. Hadjopoulos, M.D., and Reginald Burbank, M.D., New York City

IN THE class of antigenic substances the Wassermann antigen holds a unique place. Contrary to the supposed protein nature of antigenic bodies, the Wassermann antigen, the only known exception, is pure tissue lipin. It is capable of fixing complement in the presence of its specific antibody the luetic reagen, though its parenteral introduction does not give rise to specific antibody production.

Subsequent to the studies of Forssman,[1] certain specially prepared tissue extractives were taken as exhibiting true antigenic properties. Later, difficulties in producing a protein-free lipin cast doubt on the integrity of similar observations, and tended to prove that all antigens are of protein nature.

During the last year, interest in the subject was revived on the Continent: Doerr and Hallauer,[2] by the addition of protein radical, were able to change Forssman's lipoid into a full antigen. After trying various proteins, such as pig's serum, internal organs and even hemolyzed erythrocytes of rabbits they came to a belief in the real chemical combination of the lipin-protein.

A varient view was taken by Sachs and Klopstock.[3] In their opinion lipins are true antigens; but their functions as such are masked in the presence of body proteins and reappear only after the addition of the foreign protein employed, e.g., hog's serum.

By injecting Spirocheta pallida into rabbits, Klopstock[4] claims to have produced antibodies with a better selectivity for spirochete lipins in contrast to organ lipins. He concludes that the serologic changes in syphilis are induced directly by the spirochetes and indirectly through the production of lipotropic tissue antibodies in response to the specific spirochete reaction in the system.

Brandt, Guth and Muller,[5] repeated some of the above experiments and obtained only very weak fixations with the best antigens they had at the time. In a later work they found indications of organ specificity, especially with lipins extracted from brain tissue.

Much[6] who was one of the early propounders of the true antigenic nature of lipins, in a recent work admits that only certain fats can act as antigens and then only in combination with proteins. A short time age he alone claimed antigenic properties for lipins; he now is in favor of rejecting the antigenic property of *true proteins.*

While this controversy was gradually becoming acute in Europe, it occurred to us that the trend of these apparently conflicting opinions was approaching a stage that allowed a better and more intelligent explanation as to the nature of antigens in general and the Wassermann antigen in particular.

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Since chemically pure lipins are not antigenic but can be rendered so by the addition of some protein radical, whether it be pig's serum, hemolyzed erythrocytes, spirochetes or internal organs, it stands to reason that, in view of the undoubted antigenic nature of protein in general and the luetic reagen-fixing property of organ lipins in particular, the something which exhibits two serologically distinct properties (antibody-inducing and antibody-fixing) can no longer be considered as a single homogeneous body.

We took this view as a working basis in conformity with the above experimental facts, in fractioning an antigen it was only natural to ascribe to the protein fraction the immunity-exciting, *immunogenic* properties and to the lipin fraction the immune body-fixing, *immunophilic* properties. Fractioning of the antigen consisted in separating the two elements and obtaining them in the purest state possible. This was done as follows:

TECHNIC OF SPLITTING THE ANTIGEN INTO ITS IMMUNOGENIC AND IMMUNOPHILIC FRACTIONS

As it is essential to have the lipin fraction protein-free, dried and thoroughly pulverized heart muscle tissue was extracted for a week in absolute alcohol at incubator temperature, with a few daily shakings. Part of the clear supernatant fluid was removed and centrifugalized at high speed for fifteen minutes to rid of all traces of protein material held in suspension. The product thus obtained, kept at room temperature for a week, gave a white flaky-granular sediment, a mixture of fats and soaps sedimented by centrifugalization. The final product composed of fatty acids, cholesterol and lecithids represented the *immunophilic* fraction, the regular Wassermann antigen.

To obtain the immunogenic lipin-free protein fraction, a small portion from the top layer of the tissue sediment already once extracted with alcohol, was removed by means of a pipette and washed repeatedly with warm absolute alcohol until the washings were free from all traces of lipins. This was determined by pouring the entire final washing in water. In the total absence of even a trace of opalescence the extraction was considered complete. Nevertheless a further washing in equal parts of alcohol-ether mixture was given to insure the purity of the lipin-free fraction. The sediment thus obtained was dried, pulverized, and a portion of it emulsified in saline, was used for intravenous injection. Table I gives the experimental data showing the correctness of our hypothesis.

Comments.—The complement fixations were performed with all three antigens, viz., the whole heart tissue, the delipinized heart tissue (the same material that was used to immunize the animals), and the pure lipin extractive (the antigen ordinarily used for the Wassermann reaction). All three antigens were previously titrated for their antigenic, anticomplementary and hemolytic values. In the course of fixation a marked degree of hemolytic property was exhibited by the serum of the experimentally injected rabbits. This was, however, properly controlled in the standardization of the hemolytic system.

As will be seen in Table I, the delipinized heart tissue was purely im-

TABLE I

THE PRODUCTION OF LIPOTROPIC ANTIBODIES IN RABBITS BY THE PARENTERAL INTRODUCTION OF WHOLE AND DELIPINIZED OX HEART TISSUES

INJECTION AND COMPLEMENT-FIXATION TEST DATES	COMPLEMENT-FIXATION TESTS WITH ALL THREE ANTIGENS											
	WHOLE HEART ANTIG.	DELIP. HEART ANTIG.	WASSERMANN ANTIG.	WHOLE HEART ANTIG.	DELIP. HEART ANTIG.	WASSERMANN ANTIG.	WHOLE HEART ANTIG.	DELIP. HEART ANTIG.	WASSERMANN ANTIG.			
	Control Animals			Whole Heart Tissue			Experimental Animals Injected with Delipinized Tissue					
8/18/25 Preliminary Fixations	−	−	−	−	−	−	−	−	−			
8/18/25 1st Injection												
8/23/25 2nd "												
8/25/25 3rd "												
8/27/25 4th "												
8/29/25 1st Fixation	−	−	−	+	−	−	−	−	−			
9/ 5/25 2nd "	−	−	−	++	−	++	−	−	−			
9/11/25 3rd "	−	−	−	+++	−	+++	−	−	++			
9/19/25 4th "	−	−	−	+	−	−	+++	−	+++			
9/23/25 5th "	−	−	−	−	−	−	−	−	−			

TABLE II

INTENSITY AND PERCENTAGE DISTRIBUTION OF NONSPECIFIC WASSERMANN REACTIONS IN RELATION TO DISEASE

CLINICAL CONDITIONS GIVING RISE TO NONSPECIFIC REACTIONS	INTENSITY OF REACTION	GROUP PERCENTAGES
Septicemias, including Strept. Endocarditis, 15 per cent	+, ++	27 per cent
Cardiacs and cardionephritics	+	16 per cent
Anemias and Lucemias	++, +++	8 per cent
Gastric and duodenal ulcers	++	6 per cent
Malaria and lead poisoning	++	4 per cent
		61 per cent pathologic conditions
Pregnancy, normal	+	2 per cent
Sundry, normal cases	+	37 per cent
		39 per cent physiologic conditions

TABLE III

THE DISTRIBUTION OF NONSPECIFIC FIXATIONS IN FORTY-SEVEN CASES OF SUNDRY CARDIOPATHIES

CARDIAC PATHOLOGY	NUMBER OF CASES		TOTAL	PERCENTAGE POSITIVES
	POSITIVE	NEGATIVE		
Myocardiacs	4	10	14	28 per cent
Endocardiacs	9	24	33	28 per cent
Totals	13	34	47	28 per cent

munogenic and not immunophilic, i.e., it could excite the formation of specific antibody but failed to fix complement in the latter's presence. Thus a new light was shed upon the subject of antigens. Other facts of secondary importance were that the whole (nonfractioned) tissue was superior for fixation to the delipinized and pure lipin antigens. Although it seemed reasonable to ascribe this to the presence in the whole tissue of both antigenic fractions, we were inclined to think that the slight immunogenic inferiority of the delipinized antigen was not due so much to the lack of lipins in the protein molecule as to the possible chemical alteration brought about by the lipin solvents used. We have definite reasons for believing that ether alters more than alcohol. Lastly, the appearance of the reaction was more gradual than its disappearance. It took about two weeks for the reaction to appear and a little longer to disappear altogether.

ORIGIN OF THE WASSERMANN ANTIGEN

We have already mentioned the observations of Brandt, Guth, and Muller[5] as to the organ specificity exhibited by lipins and especially by those lipins extracted from brain tissue. The organ used in our experiments was ox heart. Consequently, if there is an organ specificity, lipins derived from other sources should at least not react with the same intensity to the serum of our experimental animals. In this respect our work, being but fragmentary, was not incorporated in Table I. Nevertheless, the conclusions drawn from the scant material at hand are rather in favor of such an assumption.

Landau and Held[7] observed that in thirty cases of endocarditis lenta the Wassermann reaction was positive in ten (30 per cent) and in at least

eight of these there was no other ground for suspecting specific infection. It is the opinion of the above authors that the positive reactions in these cases should be considered as incidental and secondary phenomena of a "disturbed colloidal or lipoid balance" in their serums.

It has been our experience for the last ten years that if active serum is used for the Wassermann reaction and a heart tissue plain alcoholic extract for antigen, a certain, although very small, percentage (3 per cent) of nonspecific reactions is inevitable. A clinical analysis of such reactions covering a period of six years, disclosed the fact that about 60 per cent occurred in pathologic conditions and the remaining 40 per cent in apparently normal conditions. In Table II we give the details of the percentage distribution of the nonspecific fixations.

As regards the class of cardiacs, assuming that heart tissue destruction was the cause of nonspecific reactions, we investigated the percentage occurrence of such reactions in forty-seven cases of sundry cardiopathies clinically classified as myocardiacs and endocardiacs. The findings are given in Table III.

As the percentage of fixations was equally distributed between the two major cardiac conditions, it is assumed that the rate of tissue destruction in terms of antibody production is about equal in all cardiopathies. In view of the fact that heart tissue can stimulate the production of the Wassermann reagen, the above observations point strongly to the heart as the source of the antigenic substances in syphilis, nevertheless, before we can consider seriously such a possibility, we must have a better understanding of the mode of action of other than heart tissue extractives.

It is generally accepted that heart muscle extracts are the best antigens for the Wassermann reaction, although other organ extractives have been more or less successfully used for the same purpose. It is quite possible that there may exist a certain degree of organ specificity depending on the origin of the lipin used as antigen; but, as in other immunity phenomena, the specificity is more or less generic and not strictly limited to a given organ. On the other hand, we may not exclude the possibility of a simultaneous stimulation, constructive or destructive, of more than one organ by the syphilitic virus.

As we have stated, the nonspecific reactions obtained in cardiacs were usually of a weak nature, not over two plus, while the specific titers in syphilis usually run very high. If the difference were only quantitative there would be little objection to accepting their natural similarity. As the production of antibodies depends on the effectiveness, the amount and the mode of administration of the antigen as well as the responsiveness of the recipient, it would be expecting too much to presume two clinically dissimilar diseases, syphilis and cardiopathy, to give rise to a rather specific antibody in exactly the same proportion. With all this in view we still lack direct evidence of their qualitative similarity excepting the fact that both antibodies react similarly with the same antigen.

SUMMARY AND CONCLUSIONS

1. Antigens cannot be considered as homogeneous material.

2. They are composed of two serologically dissimilar molecules: (a) the *immunogenic,* i.e., immunity-exciting or antibody-producing fraction, a prop-

erty residing in the protein molecule, and (b) the *immunophilic*, i.e., the antibody-fixing property residing in the lipin molecule.

3. This theory applied to the heart muscle tissue proved correct by artificially inducing the Wassermann reagen in experimental animals by the parenteral introduction of the immunogenic fraction and the subsequent fixation of the latter by the immunophilic lipin fraction.

4. The serologic changes in the course of syphilitic infection can be induced by widely dissimilar agents affecting similar organs or groups or organs and essentially the myocardium.

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THE DUALITY OF THE ANTIGENIC NATURE OF ERYTHROCYTES

By L. G. Hadjopoulos, M.D., and Reginald Burbank, M.D., New York City

In a previous communication[1] we demonstrated the existence of two distinct antigenic fractions in the heart muscle tissue. First the *immunogenic* (immunity-exciting) or antibody-forming fraction represented in the protein molecule, and second, the *immunophilic* (the immune element) or antibody-fixing fraction represented in the lipin molecule derived from the heart muscle tissue by alcoholic extraction.

The same technic was followed in the present work that was applied in fractioning the heart muscle antigen. In short, it consisted in washing the erythrocytes with saline until they were absolutely free of serum proteins. The dried and pulverized material was extracted with absolute alcohol. After decanting the alcoholic, immunophilic fraction, the sediment was repeatedly washed in a mixture of equal volumes of alcohol and ether until the sediment was absolutely lipin free. This end product, dried and pulverized, constituted the immunogenic fraction of the erythrocytic antigen.

Rabbits were injected separately with a saline suspension of the above protein and lipin fractions. They were given in all five progressively increasing doses within a period of fifteen days. On the tenth day after the last dose they were bled. The results of their serum reactions are given in Table I.

Table I

Immunity Reaction Titers against Fractional Erythrocytic Antigen Injections

Description	Agglutinin titers	Hemolysin titers	Complement-fixations with	
			Lipin fraction	Delipinized protein fraction
Rabbit A control animal	1: 1	1: 50	–	–
Rabbit 1 injected prot. fraction	1:10	1:5,000	++++	–
" 2 " " "	1: 5	1:3,000	++++	–
" 3 " lipin "	1: 2	1: 25	–	–
" 4 " " "	1: 1	1: 50	–	–

Independent of whether the injected material was heart muscle tissue or erythrocyte, the nature of findings was practically identical. The simultaneous stimulation of other immune elements as agglutinins and hemolysins, was similarly expected. In a previous article[2] we demonstrated the production of hemolysins by injecting dehemoglobinized erythrocytes (cell stromata) and that of agglutinins, by injecting cell-free hemoglobin. The only departure in the present work was the use of delipinized cells (stroma-plus hemoglobin) to prove that the lipin element was not necessary for the production of immune bodies.

As in our findings in the case of heart muscle tissue, the antibody formed in response to delipinized cell protein injection was strongly lipotropic and com-

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pletely nonproteotropic. At this stage of the work the question came up, considering its mode of production, as to whether or not this antibody could display any specificity. The direct experimental evidence, as given in Table II, is in favor of such specificity.

The above experiments clearly indicate the specific nature of cell lipins in contrast to heart tissue lipins, i.e., the Wassermann antigen. Moreover this specificity is not absolute as we demonstrate in Table III, where Wassermann positive serums were tested with both antigens.

Comments.—In the light of our previous findings in reference to the specificity of lipin antigens of different origins, the above tests are rather confusing unless we consider certain facts. First, the titration of our cell lipin antigen

TABLE II

RESULTS OF COMPLEMENT-FIXATION TESTS OF IMMUNE BODIES PRODUCED BY ERYTHROCYTIC PROTEIN INJECTIONS WITH THEIR HOMOLOGOUS CELL-LIPIN ANTIGEN AND THE HETEROLOGOUS WASSERMANN LIPIN ANTIGEN

DESCRIPTION	AMOUNT SERUM C.C.	COMPLEMENT-FIXATIONS WITH CELL LIPIN ANTIGEN	COMPLEMENT-FIXATIONS WITH WASSERMANN ANTIGEN
Complement control (Pooled guinea pig serum)	0.04	–	–
Serum of rabbit injected with delipinized erythrocytic protein	0.0001	++	–
	0.0002	+++	–
	0.0004	++++	–

The cell lipin antigen was titrated previously against one unit of complement. The lowest dilution that did not interfere with hemolysis was taken as the unit (in this particular case 0.50 c.c. of 1:40 dilution). Two units of complement were used in the tests.

TABLE III

THE NATURAL ANTISHEEP HEMOLYSIN TITERS OF HUMAN SERA AND THEIR EFFECT ON COMPLEMENT-FIXATION REACTIONS WITH CELL LIPIN ANTIGEN AND WASSERMANN ANTIGEN

SERIAL NUMBER	DESCRIPTION	NATURAL HEMOLYSIN TITERS	COMPLEMENT-FIXATION TESTS WITH CELL LIPIN ANTIGEN	COMPLEMENT-FIXATION TESTS WITH WASSERMANN ANTIGEN
	Complement control (Pooled guinea pig serum)	None	–	–
1316	Wassermann negative controls	0.02 c.c.	++	–
1317	" " "	0.02 c.c.	++	–
1318	" " "	0.02 c.c.	+	–
1319	" " "	0.025 c.c.	–	–
1320	" " "	0.02 c.c.	+	–
1321	" " "	0.02 c.c.	++	–
D. M.	" " "	0.025 c.c.	–	–
D. S.	" " "	0.03 c.c.	–	–
N. K.	Luetic under treatment	0.03 c.c.	+++	++++
J. P.	" " "	0.02 c.c.	++++	++++
1619	" " "	0.02 c.c.	++++	++++

was performed against guinea pig serum which is invariably devoid of antisheep hemolysin. Second, human serum invariably contains antisheep hemolysin in fair amounts. It was therefore natural to expect a certain degree of fixation of complement in the presence of a homologous antigen, the cell lipin.

By comparing the natural hemolysin titer of the above serums against their homologous fixation, we found that the titer of our cell lipin antigen, 0.50 c.c. of a 1:40 dilution, was just sufficient to detect such concentrations of natural hemolysin as would occur in 0.02 c.c. of serum or less. Lower con-

centrations were not detected with this standard dose. (In Table III compare cases 1316, 1317, 1318, 1320, and 1321, against 1319, D. M., and D. S.) In the luetic series, with the cell lipin antigen case N. K. should be negative and cases J. P. and 1619 not over two plus. As they give stronger fixations than would ordinarily be attributed to the amount of natural hemolysin present, however, the question arises as to whether we should consider this as the nonspecific influence of a strongly concentrated luetic reagen on a weak sister antibody.

As such phenomena are common in serologically allied substances we resorted to the absorption method of separating and handling them individually. In default of a method to eliminate the luetic reagen, we absorbed the natural hemolysin with sheep cells and tested the cell lipin antigen against the luetic serums minus the hemolysin. The results are given in Table IV.

Comments.—With the complete absorption of the natural hemolysin (the antibody to sheep cell lipin) the complement fixation with the latter antigen was reduced to negative while the Wassermann reaction was unaffected. As a matter of fact, absorption slightly increased the Wassermann reaction.

TABLE IV

THE EFFECT OF THE ELIMINATION OF NATURAL ANTISHEEP HEMOLYSIN ON THE CELL LIPIN AND THE WASSERMANN REACTION

SERIAL NUMBER	DESCRIPTION	NATURAL HEMOLYSIN TITERS	COMPLEMENT-FIXATION TESTS WITH CELL LIPIN ANTIGEN	COMPLEMENT-FIXATION TESTS WITH WASSERMANN ANTIGEN
J. P.	Luetic, unabsorbed serum	0.02 c.c.	++++	++++
	absorbed with cells	None	–	++++
1606	Luetic, unabsorbed serum	0.02 c.c.	++	++
	absorbed with cells	None	–	+++
1692	Chronic, unabsorbed serum	0.01 c.c.	++	+
	Cardiac, absorbed with cells	None	–	+

It is generally accepted that the presence of a high hemolysin titer in a luetic serum may result in a relative reduction of the intensity of the Wassermann reaction. Our findings, which are not limited to the few cases above tabulated are in conflict with this view. We have no satisfactory explanation for this except the possibility that during the absorption of the natural hemolysin, the addition of sheep cells introduces a nonspecific element that reacts the same way as the luetic reagen.

In other respects the findings were as would be expected, namely, the degree of cell lipin fixation was in proportion to the natural hemolytic titers (Cases 1606, 1692) and the presence of synergism between the luetic reagen on the cell lipin antigen as in case J. P. Case 1692 was expressly introduced in this table as a group representative of some minor nonspecific fixations occurring in a fair percentage of chronic cardiacs, with the purpose of investigating whether such minor nonspecific Wassermann reactions were due to a reversal of the synergism between the sister antigens. Basing our stand on this experiment and others that are not quoted here, we may safely conclude that the weak fixations occurring in cardiacs are not at all influenced by the presence of natural hemolysins but that they are specific, indicating in

a way heart tissue degeneration. This aspect of the question has already been dealt with in a previous communication.[1]

SUMMARY AND CONCLUSIONS

In a previous communication we demonstrated the existence of two distinct antigenic fractions in the heart muscle tissue; the *Immunogenic* (immunity exciting) or antibody-forming fraction, a property limited to the protein molecule, and the *Immunophilic* (immune element) or antibody-fixing fraction, a property of the lipin molecule derived from the same tissue. The same principle was applied in the case of sheep's erythrocytes and found strictly correct, thus demonstrating the dual nature of similar antigens.

The hemolysin, an antibody, whether produced artificially in animals or naturally present in human serums, immunologically is identical with the lipotropic antibody produced against the immunogenic fraction of red cells.

This lipotropic antibody is strictly specific, like its sister antibody produced by the parenteral introduction of the immunogenic fraction of heart muscle tissue. The coexistence of both sister antibodies, the natural hemolysin and the Wassermann reagen, displays certain synergistic relationship. The synergism, however, is not reversible. In the presence of both antibodies the natural hemolysin alone is stimulated by the Wassermann reagen, the reverse never taking place. Therefore, the weak nonspecific Wassermann reactions that occur in some cases of cardiacs and cardionephritics indicate the existence of a specific lipotropic antibody produced in response to the stimulus of the immunogenic fraction of heart muscle tissue.

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STUDIES IN LOCAL ANESTHESIA, VI

Further Observations of Para-Amino-Benzoate Compounds on the Rabbit Eye*

By Seymour J. Cohen, M.S., M.D., Chicago, Ill.

In a previous paper,† we reported the action of a series of para-amino-benzoate compounds in producing local anesthesia on the eyes of rabbits. These preparations were prepared by Dr. Roger Adams of the University of Illinois. He has prepared and furnished us with another series of drugs, which are also based on the structural formula of procaine and for which we have also determined the anesthetic efficiency in producing anesthesia on the rabbit eye.

In the tables are the series of drugs submitted to us for examination.

METHOD

The drugs were prepared in mol./20 concentration, which corresponds to about 1.7 per cent cocaine solution. The method was the same as used in the previous work,—chiefly the instillation of the anesthetic solution into the conjunctival sac of a rabbit for one minute, then determining the duration of anesthesia as shown by the action of the winking reflex on touching the corner with an applicator.

These results indicate that the greatest anesthetic efficiency for this series of drugs occurs when the terminal amino group contains either the ethyl, propyl, or butyl radicals. The introduction of the methyl radical in this terminal amino group causes a loss of the anesthetic action of this drug (No. 14), while the introduction of the amyl radical produces a drug that is very irritant and sometimes corrosive when compared with the same concentration of a cocaine solution (Nos. 9, 10, 17, 20). The substitution of the piperidine ring in place of the terminal amino group renders the drug much less anesthetic (Nos. 7, 8, 15, 16). The substitution of the cyclohexane radical for the alcohol portion of the molecule produces a drug with strong anesthetic properties (Nos. 22, 23, 24, 26, 28). The one phosphate salt is much less anesthetic than the hydrochloride salt. (1A) Judging from our one example, the cis-transisomerism (Nos. 22 and 23) has no influence on the anesthetic value of the drug. Both are good anesthetics.

It appears that the drugs with the greatest anesthetic value and the least corrosive action are those with the normal propyl and butyl radical (No. 18) in the terminal amino group. The iso and secondary salts of these

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TABLE I

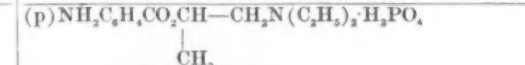
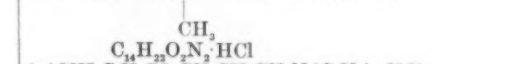
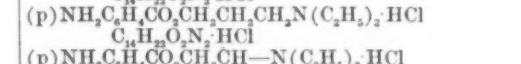
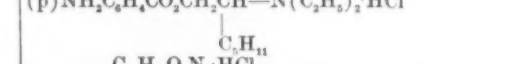
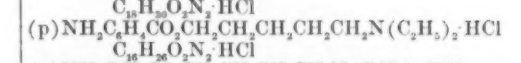
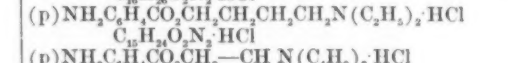
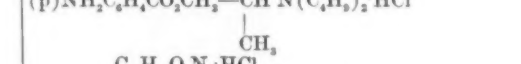
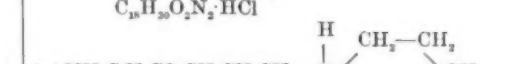
NAME	FORMULA	MOL. WT.
1.A. β-Diethylamino isopropyl para-amino-benzoate phosphate		348
1. β-Diethylamino isopropyl para-amino-benzoate hydrochloride		286
2. β-Diethylamino-n-propyl para-amino-benzoate hydrochloride		286
3. β-Diethylamino-n-heptyl para-amino-benzoate hydrochloride		342
4. ε-Diethylamino-n-amyl para-amino-benzoate hydrochloride		314
5. δ-Diethylamino-n-butyl para-amino-benzoate hydrochloride		300
6. β-Di-n-butyl amino-n-propyl para-amino-benzoate hydrochloride		342
7. γ (3-carbomethoxy piperidyl)n-propyl para-amino-benzoate hydrochloride		356
8. γ-Piperidyl-n-propyl para-amino-benzoate hydrochloride		298
9. γDi-iso-amylamino propyl para-amino-benzoate hydrochloride		370

TABLE I—CONT'D

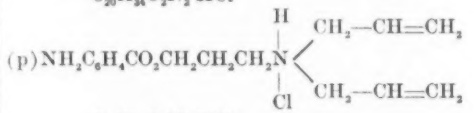
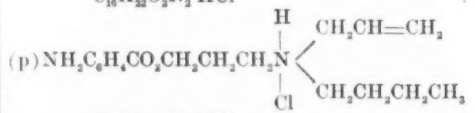
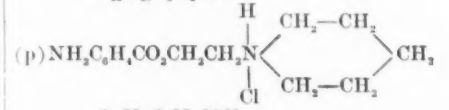
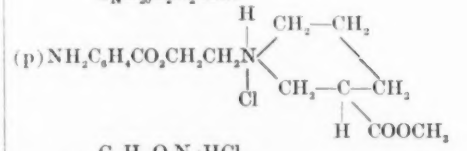
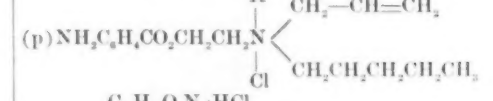
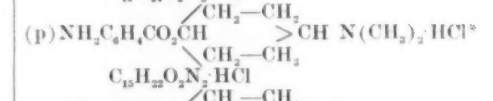
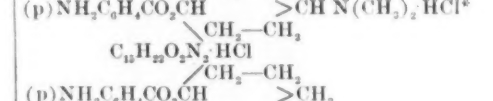
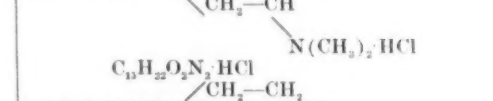
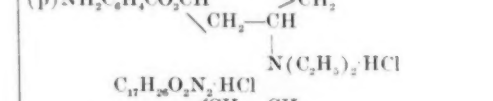
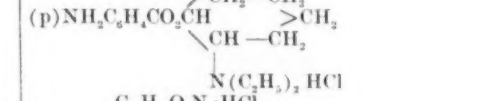
NAME	FORMULA	MOL. WT.
10. γ-Di-n-amylamino-propyl para-amino-benzoate hydrochloride	$(p)NH_2C_6H_4CO_2CH_2CH_2CH_2N(C_5H_{11}(n))_2HCl$ $C_{20}H_{34}O_2N_2 \cdot HCl$	370
11. γ-Di allylamino propyl para-amino-benzoate hydrochloride	 $C_{16}H_{22}O_2N_2 \cdot HCl$	310
12. γ-Allyl-n-butylamino propyl para-amino-benzoate hydrochloride	 $C_{17}H_{26}O_2N_2 \cdot HCl$	326
13. γ-Di-sec-butylamino propyl para-amino-benzoate hydrochloride	$(p)NH_2C_6H_4CO_2CH_2CH_2CH_2N(C_4H_9(sec))_2 \cdot HCl$ $C_{15}H_{30}O_2N_2 \cdot HCl$	342
14. γ-Dimethylamino propyl para-amino-benzoate hydrochloride	$(p)NH_2C_6H_4CO_2CH_2CH_2CH_2N(CH_3)_2HCl$ $C_{12}H_{18}O_2N_2 \cdot HCl$	258
15. β-Piperidyl ethyl para-amino-benzoate hydrochloride	 $C_{14}H_{20}O_2N_2 \cdot HCl$	284
16. β (3-carbomethoxy piperidyl) ethyl para-amino-benzoate hydrochloride	 $C_{16}H_{22}O_4N_2 \cdot HCl$	342
17. β-Di-iso-amylamino ethyl para-amino-benzoate hydrochloride	$(p)NH_2C_6H_4CO_2CH_2CH_2N(C_5H_{11}(iso))_2HCl$ $C_{19}H_{32}O_2N_2 \cdot HCl$	356
18. β-Di-n-butylamino ethyl para-amino-benzoate hydrochloride	$(p)NH_2C_6H_4CO_2CH_2CH_2N(C_4H_9(n))_2HCl$ $C_{17}H_{28}O_2N_2 \cdot HCl$	328

TABLE I—CONT'D

NAME	FORMULA	MOL. WT.
19. β-Di sec-butylamino ethyl para-amino-benzoate hydrochloride	(p)$NH_2C_6H_4CO_2CH_2CH_2N(C_4H_9(sec))_2HCl$ $C_{17}H_{29}O_2N_2 \cdot HCl$	328
20. β-Di-n-amylamino ethyl para-amino-benzoate hydrochloride	(p)$NH_2C_6H_4CO_2CH_2CH_2N(C_5H_{11}(n))_2HCl$ $C_{19}H_{33}O_2N_2 \cdot HCl$	356
21. β-Allyl-n-butylamino ethyl para-amino-benzoate hydrochloride	 $C_{16}H_{24}O_2N_2 \cdot HCl$	312
22. 4-Dimethylamino cyclohexyl para-amino-benzoate hydrochloride	 $C_{15}H_{22}O_2N_2 \cdot HCl$	298
23. 4-Dimethylamino cyclohexyl para-amino-benzoate hydrochloride	 $C_{15}H_{22}O_2N_2 \cdot HCl$	298
24. 3-Dimethylamino cyclohexyl para-amino-benzoate hydrochloride	 $C_{15}H_{22}O_2N_2 \cdot HCl$	298
26. 3-Dimethylamino cyclohexyl para-amino-benzoate hydrochloride	 $C_{17}H_{26}O_2N_2 \cdot HCl$	326
28. 2-Dimethylamino cyclohexyl para-amino-benzoate hydrochloride	 $C_{17}H_{26}O_2N_2 \cdot HCl$	326

*Stereoisomeric forms. No. 22 has a melting point of 220°-222° C., while No. 23 melts at from 263°-264° C.

TABLE II

DRUG	DURATION OF ANESTHESIA IN MIN.	MOL. WT.	REMARKS
1. β-Diethylamino isopropyl para-amino-benzoate hydrochloride	28	286	
1.A. β-Diethylamino isopropyl para-amino-benzoate phosphate	8	348	Poor
5. δ-Diethylamino-n-butyl para-amino-benzoate hydrochloride	30	300	
4. ε-Diethylamino-n-amyl para-amino-benzoate hydrochloride	36	314	
3. β-Diethylamino-n-heptyl para-amino-benzoate hydrochloride	31	342	Irritant
14. γ-Dimethylamino propyl para-amino-benzoate hydrochloride	0	258	Poor
2. β-Diethylamino-n-propyl para-amino-benzoate hydrochloride	36	286	
11. γ-Di allyl amino propyl para-amino-benzoate hydrochloride	28	310	
21. β-Allyl-n-butylamino ethyl para-amino-benzoate hydrochloride	37	312	
12. γ-Allyl-n-butylamino propyl para-amino-benzoate hydrochloride	28	326	
18. β-Di-n-butylamino ethyl para-amino-benzoate hydrochloride	29	328	
19. β-Di sec-butylamino ethyl para-amino-benzoate hydrochloride	33	328	Irritant
6. β-Di-n-butyl amino-n-propyl para-amino-benzoate hydrochloride	45	342	Irritant
13. γ-Di-sec-butylamino propyl para-amino-benzoate hydrochloride	49	342	Irritant
17. β-Di-iso-amylamino ethyl para-amino-benzoate hydrochloride	75	356	Corrosive
20. β-Di-n-amylamino ethyl para-amino-benzoate hydrochloride	75	356	Corrosive
9. γ-Di-iso-amylamino propyl para-amino-benzoate hydrochloride	75	370	Corrosive
10. γ-Di-n-amylamino propyl para-amino-benzoate hydrochloride	75	370	Corrosive
15. β-Piperidyl ethyl para-amino-benzoate hydrochloride	10	284	Poor
16. β (3-carbomethoxy piperidyl) ethyl para-amino-benzoate hydrochloride	0	342	Poor
8. γ-Piperidyl-n-propyl para-amino-benzoate hydrochloride	28	298	
7. γ (3-carbomethoxy piperidyl) n-propyl para-amino-benzoate hydrochloride	0	356	Poor
22. 4-Dimethylamino cyclohexyl para-amino-benzoate hydrochloride	43	298	
23. 4-Dimethylamino cyclohexyl para-amino-benzoate hydrochloride	44	298	
24. 3-Dimethylamino cyclohexyl para-amino-benzoate hydrochloride	22	298	
26. 3-Dimethylamino cyclohexyl para-amino-benzoate hydrochloride	42	326	Irritant
28. 2-Dimethylamino cyclohexyl para-amino-benzoate hydrochloride	21	326	

radicals are also good anesthetics but have a tendency to cause irritation and edema of the conjunctiva. (Nos. 6, 13, 19.)

I wish to thank Dr. Jacob Sacks and Mr. M. Cahan for their help in this work.

A NOTE UPON THE BACTERIOLOGY OF EXCISED TONSILS*

By Robert A. Kilduffe,† A.M., M.D., Assisted by W. W. Hersohn,
Atlantic City, N. J.

WHILE it is not the presence of bacteria but the sequelae consequent upon their successful invasion of the body tissues which produce the phenomena of disease, and while the tonsils are well recognized as the habitat of a varied bacterial flora, in view of their implied and frequently proved importance as foci of infection this study of the bacteriology of excised tonsils was deemed of interest.

The tonsils were received in the laboratory wrapped in sterile gauze. After searing the outer surface, streak cultures were made upon blood agar plates from which, when necessary, subcultures were made to various media for bacterial identification.

In all a total of 409 tonsils were thus examined with the results tabulated below:

Table I

Pure Cultures

ORGANISM	NUMBER OF TIMES FOUND	
Pneumococcus (mucosus capsulatus)	3	
Pneumococcus (other types)	21	
M. catarrhalis	9	
B. Friedländer	4	
Staphylococcus albus	11	
Staphylococcus aureus	32	
Streptococcus hemolyticus	2	
Streptococcus nonhemolyticus	5	87

As all the tonsils examined were definitely diseased and the site of chronic lesions, the incidence of the various organisms is of some interest. The rather low incidence of streptococci (6.4 per cent) was somewhat surprising, the organisms most frequently found (Staphylococcus aureus, 33 per cent; M. catarrhalis, 34 per cent; and pneumococcus, 54 per cent) being organisms not usually associated with those diseases often attributed to focal tonsillar infections.

In view of the fact that the tonsils in general were of chronic type, the

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infrequency of pure cultures is not surprising, secondary invasions being common.

The varied bacterial flora encountered emphasizes the difficulty associated with the interpretation of tonsillar cultures in the study of focal infections and also, perhaps, suggests the advisability of the routine use of the blood agar plate in conjunction with the Loeffler tube in the study of throat infections.

TABLE II

TWO ORGANISMS IN CULTURE

ORGANISMS	NUMBER OF TIMES FOUND	
Streptococcus nonhemolyticus and pneumococcus	16	
Streptococcus nonhemolyticus and Staphylococcus albus	13	
Streptococcus nonhemolyticus and Staphylococcus aureus	13	
Streptococcus nonhemolyticus and M. catarrhalis	5	
Streptococcus nonhemolyticus and leptothrix	1	
Streptococcus nonhemolyticus and Gram-negative bacillus, unidentified	3	
Streptococcus nonhemolyticus and B. Friedländer	3	54
Streptococcus hemolyticus and Staphylococcus aureus	2	
Streptococcus hemolyticus and pneumococcus	4	
Streptococcus hemolyticus and M. catarrhalis	1	
Streptococcus hemolyticus and leptothrix	1	
Streptococcus hemolyticus and Gram-negative bacillus, unidentified	1	9
Streptococcus viridans and Staphylococcus albus	2	
Streptococcus viridans and pneumococcus	2	
Streptococcus viridans and M. catarrhalis	2	6
Pneumococcus and Staphylococcus albus	20	
Pneumococcus and Staphylococcus aureus	24	
Pneumococcus and M. catarrhalis	36	
Pneumococcus and diphtheroids	1	
Pneumococcus and B. influenzae	4	
Pneumococcus and leptothrix	1	
Pneumococcus and Gram-negative bacillus, unidentified	19	
Pneumococcus and Gram-positive bacillus, unidentified	1	106
Staphylococcus aureus and Staphylococcus albus	14	
Staphylococcus aureus and M. catarrhalis	1	
Staphylococcus aureus and Gram-negative bacillus, probably B. Friedländer	6	
Staphylococcus aureus and Gram-positive bacillus, unidentified	1	
Staphylococcus aureus and B. proteus vulgaris	1	23
Staphylococcus albus and B. Friedländer	5	
Staphylococcus albus and Gram-negative bacillus, unidentified	3	
Staphylococcus albus and B. influenzae	1	
Staphylococcus albus and M. catarrhalis	8	
Staphylococcus albus and diphtheroids	1	18
M. catarrhalis and diphtheroids	1	
M. catarrhalis and B. Friedländer	6	
M. tetragenus and B. Friedländer	3	10

TABLE III

THREE ORGANISMS IN CULTURE

ORGANISMS	NUMBER OF TIMES FOUND	
Streptococcus viridans, Streptococcus nonhemolyticus and Staphylococcus aureus	1	
Streptococcus hemolyticus, pneumococcus, and Staphylococcus aureus	2	
Streptococcus nonhemolyticus, pneumococcus, and leptothrix	1	
Streptococcus nonhemolyticus, pneumococcus, and diphtheroids	1	
Streptococcus nonhemolyticus, pneumococcus, and M. catarrhalis	7	
Streptococcus nonhemolyticus, pneumococcus, and Staphylococcus aureus	10	
Streptococcus nonhemolyticus, pneumococcus, and Staphylococcus albus	1	
Streptococcus nonhemolyticus, pneumococcus, and B. Friedländer	1	
Streptococcus nonhemolyticus, M. catarrhalis, and leptothrix	1	
Streptococcus nonhemolyticus, M. catarrhalis, and diphtheroids	1	
Streptococcus nonhemolyticus, M. catarrhalis, and Gram-positive bacilli, unidentified	1	
Streptococcus nonhemolyticus, Staphylococcus aureus, and Gram-positive bacilli, unidentified	2	
Streptococcus nonhemolyticus, Staphylococcus aureus, and diphtheroids	1	
Streptococcus nonhemolyticus, Staphylococcus albus, and B. Friedländer	4	
Streptococcus nonhemolyticus, Staphylococcus aureus, and B. Friedländer	5	
Pneumococcus, Staphylococcus aureus, and B. Friedländer	5	
Pneumococcus, M. catarrhalis, and B. Friedländer	17	
Pneumococcus, M. catarrhalis, and leptothrix	2	
Pneumococcus, M. catarrhalis, and diphtheroids	6	
Pneumococcus, M. catarrhalis, and Staphylococcus aureus	10	
Pneumococcus, M. catarrhalis, and Staphylococcus albus	5	
M. catarrhalis, Staphylococcus aureus, and Gram-negative bacillus, unidentified	2	
M. catarrhalis, Staphylococcus aureus, and diphtheroids	1	87

TABLE IV

FOUR ORGANISMS IN CULTURE

ORGANISMS	NUMBER OF TIMES FOUND	
Streptococcus nonhemolyticus, Staphylococcus aureus, M. catarrhalis, Gram-negative bacillus, unidentified (probably B. Friedländer)	3	
Streptococcus nonhemolyticus, pneumococcus, M. catarrhalis, Gram-negative bacillus, unidentified (probably B. Friedländer)	2	
Pneumococcus, diphtheroids, M. catarrhalis, and Staphylococcus aureus	3	
Pneumococcus, Staphylococcus albus, M. catarrhalis and leptothrix	1	9
Total cultures	409	

TABLE V

PERCENTAGE INCIDENCE OF BACTERIA FOUND

ORGANISM	NUMBER TIMES FOUND	PER CENT
Streptococcus viridans	7	1.4
Streptococcus hemolyticus	13	3.0
Streptococcus nonhemolyticus	93	2.0
Leptothrix	8	0.9
M. catarrhalis	142	34.0
Diphtheroids	15	3.0
B. influenzae	5	1.0
Pneumococcus	224	54.0
B. Friedländer	61	14.0
B. proteus	1	0.2
Staphylococcus aureus	136	33.0
Staphylococcus albus	7	1.7
Gram-positive, unidentified bacilli	4	0.9
Total	757	

THE ACTION OF INDOL AND SKATOL ON THE HEART*

By J. A. Waddell, M.D., and J. A. Calhoun, A.B., Charlottesville, Va.

INDOL and skatol are described in works on biologic chemistry and physiology as being poisonous protein derivatives which are formed in the intestine by bacterial action. To them is attributed the characteristic odor of the feces. On absorption from the alimentary canal, they are normally detoxicated, in the liver, by conjugation with certain metabolites such as sulphuric and glycuronic acid.

They are referred to in clinical literature as factors in the causation of the symptoms observed in intestinal stasis, wound suppuration, and focal infection. While the part they play in such conditions is not definitely established, the fact that they are present coupled with the evidence that they are toxic makes a strong case against them.

The most outstanding investigations of their physiologic effects are those of Rovighi[1] on indol and skatol, of Herter[2] on indol, and of Salant and Kleitman[3] on skatol. The first mentioned experimented on rabbits and other mammals and noted, among various effects, feeble heart action. The second author observed in dogs and rabbits respiratory depression, general prostration, and circulatory depression. Salant and Kleitman, using cats and dogs, reported central nervous system and circulatory depression, the latter tending to persist in the case of the dog.

All of the above employed intact animals and dealt with the grosser features of the intoxication. Though each reported depression of the circulation, they noted coincidently changes in the nervous apparatus and in respiration, as well as in other systems. Which effects were primary and which were secondary was not worked out. None of them examined the heart as a separate entity nor did they definitely establish a cardiac action on the part of these substances, although Herter does cite an experiment in which the death of the experimental animal "seemed" to be cardiac.

In view of the rather fragmentary state of the knowledge regarding the effects of indol and skatol on specific organs, particularly the heart, we have undertaken this investigation of their cardiac actions, individually and in combination in various proportions, on cold and warm blooded animals. Our study is limited to the cardiac effects, uncomplicated by changes in the blood vessels, the nervous system, or the respiration.

*From the Pharmacological Laboratory of the Medical School of the University of Virginia.

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METHODS AND MATERIALS

The freshly excised hearts of frogs, turtles, cats, and rabbits were used in these experiments. The tracings were taken from the apex. A slightly different procedure was necessary for the cold blooded as compared with the warm.

In the case of the frogs and turtles, the procedure for perfusing was the same as that employed in a previous paper.[4] It consisted, in brief, in admitting the perfusate through the vena cava and securing the outflow from the aorta. A uniform temperature and constant pressure were maintained. The

Fig. 1.—Tracing 1. Heart of turtle, showing the effect of half saturated indol with partial recovery after removal.

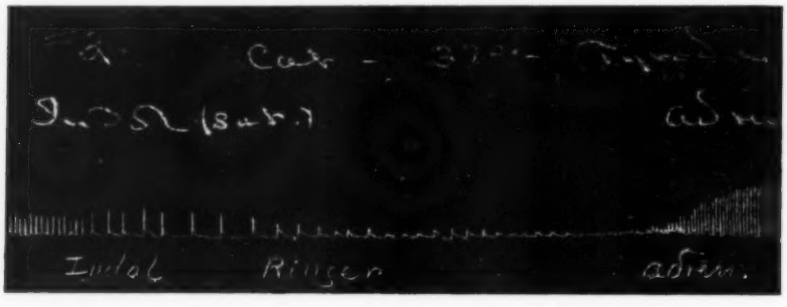

Fig. 2.—Tracing 2. Heart of cat, showing the effect of saturated indol with antagonism by epinephrine.

study was begun in the early fall and continued into the winter. The animals were kept in the laboratory for twelve hours before the experiment, in order not to be subjected to a sudden change in temperature just prior to the observations. Ringer's (Howell) solution was employed, at room temperature 20-22° C. and at 27° C.

The hearts of cats and rabbits were perfused with Tyrode's solution at 37° C. A modified Langendorff method was employed, the perfusate being admitted through the tip of the left auricle and the outflow secured from the aorta. The right auricle was punctured to permit its emptying fluid received through the coronary circulation.

As is well known, indol and skatol are very slightly soluble. Chemical tables of solubilities do not state the degree.[5] Hence, the strengths of the solutions we used cannot be expressed in per cent; accordingly, we have employed the terms, "saturated solution," "half-saturated," etc., to indicate the comparative concentrations, which of necessity were extremely dilute.

The following brands of the drugs were employed: Skatol from Eimer & Amend, Kahlbaum, and Eastman; Indol from Eimer & Amend, Theo. Schuchardt and Kahlbaum. One Kahlbaum and the Suchart preparation were old and quantitatively less effective; otherwise all acted alike.

Our solutions were prepared twelve hours in advance by adding the drug in excess to Ringer's or Tyrode's solution and applying a temperature of

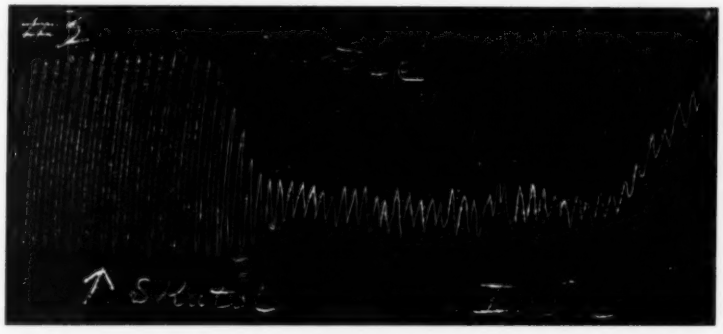

Fig. 3.—Tracing 3. Heart of turtle, showing the effect of half saturated skatol with antagonistic action of indol.

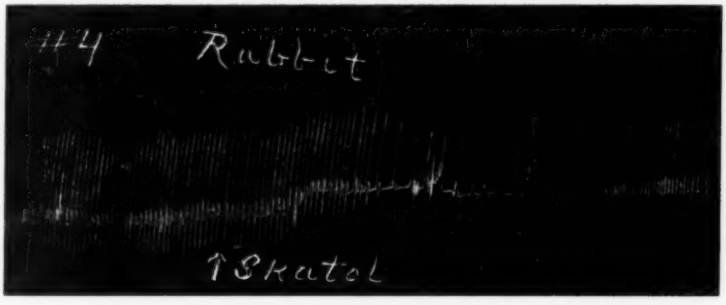

Fig. 4.—Tracing 4. Heart of rabbit, showing the effect of saturated skatol with recovery on removal.

60° C. for fifteen minutes. These saturated solutions were then filtered—that in Ringer's at room temperature and that in Tyrode's at 37° C. From the filtrate the working dilutions were made.

EXPERIMENTAL DATA

The experimental data will be presented under the following captions: (1) Indol, (2) Skatol, (3) Indol and Skatol with Other Drugs, (4) Indol and Skatol with One Another, and (5) Indol and Skatol on Other Nonvoluntary Muscle.

1. *Indol.*—On the hearts of the turtle and frogs, all strengths of indol down to one-eighth saturated solutions were effective in slowing the heart

and in decreasing the output per minute and per beat. The latent period was relatively short. The lowest concentrations produced chiefly slowing; intermediate ones, slowing with usually a gradual decrease in diastole and occasionally grouped beats; and the highest, arrest, usually systolic, which was preceded by a few extrasystoles. The auricle stopped before the ventricle. The arrested heart responded to mechanical and electric stimuli and to epinephrine. On perfusion with plain Ringer's fluid, the effects of indol were almost entirely abolished. Automaticity was reestablished in the quiescent with peristaltic phenomena very pronounced initially. The force of the contractions was not restored. The A-V interval was not changed and heart-

Fig. 5.—Tracing 5. Heart of turtle, showing the effect of a balanced mixture of indol and skatol with recovery on removal.

Fig. 6.—Tracing 6. Intestine of rabbit, showing the effect of half saturated indol with partial recovery on removal.

block was not exhibited. Quantitatively, the effects were more marked at 27° C. than at 20° C. and on reapplication than initially.

On the hearts of cats and rabbits, solutions saturated at 27° C. produced almost immediate arrest of both chambers in diastole, usually; one-half saturated, only slowing with decreased amplitude. Withdrawal of the drug was rapidly followed by almost complete restoration. The decreased relaxation, observed in the turtle, was not exhibited in the case of the warm-blooded heart and systolic arrest was but rarely observed.

2. *Skatol.*—On the heart of the frogs and turtles, all strengths of skatol down to one-eighth saturated solutions were effective in slowing the heart

and decreasing the output. The latent period was long compared with that of indol. The lowest concentrations produced slowing; intermediate ones, slowing with usually a gradual decrease in systole; and the highest, arrest, which was usually diastolic. The auricle stopped before the ventricle and its cessation was preceded by marked dilatation. The arrested heart responded to electric and mechanical stimulation and to epinephrine. Prolonged perfusion with plain Ringer's restored the rhythm but not the force of the contractions. The drug was more effective on reapplication. The A-V interval was not altered and heart-block was not observed. The drug was somewhat more effective at 27° C. than at 20° C. as was also the case with indol.

On the hearts of cats and rabbits, solutions saturated at 37° C. produced tardily an arrest of both chambers in diastole; one-half saturated solutions,

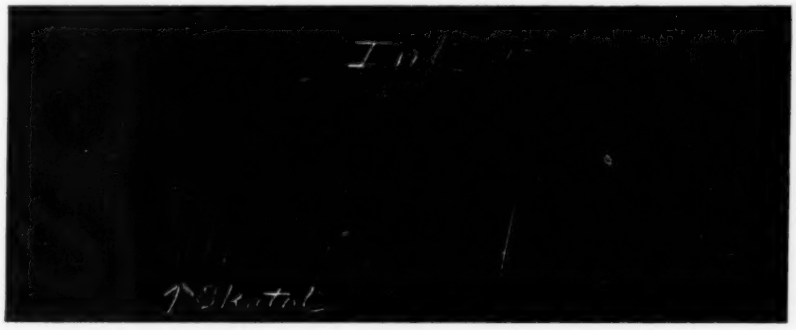

Fig. 7.—Tracing 7. Intestine of rabbit, showing the effect of saturated skatol with partial recovery on removal.

Fig. 8.—Tracing 8. Heart of frog, showing the effect of isatin with recovery on removal.

only slowing with decreased amplitude, less than after indol. The decrease in the height of the concentrations, observed in the turtles, was also shown in the warm-blooded hearts.

3. *Indol and Skatol with Other Drugs.*—Possible interactions with epinephrine and atropine were investigated. The former was antagonistic, the result being the algebraic sum. Atropinizing did not prevent nor abolish their actions or qualitatively alter them.

4. *Indol and Skatol with One Another.*—The effects of indol and skatol have been shown to be very similar. This was to be expected from a consideration of their close chemical relationships. The most noteworthy difference is in there usually being exhibited in cold-blooded hearts after indol a

decrease in relaxation and after skatol a decrease in contraction. In view of this it would seem that they might be antagonistic in some respects, at least on the amphibian heart. Hence, they were employed in the following further experiments on the turtle (a) in sequence and (b) mixed together in various concentrations.

(a) In sequence. On changing immediately for instance from indol to skatol, the diastolic shortening of the former was altered to the systolic shortening of the latter. The effect was always that of the drug last used, though somewhat reduced. The change was exhibited sharply; no intermediate balancing of effects was observable. (See Fig. 3.)

(b) Mixed together. Since indol and skatol are not nicely quantitative in their effects, the endeavor was made, by trial on individual hearts, to mix them in balanced proportions; i.e., if skatol one-fourth saturated produced approximately the same decrease in systole as indol one-fourth did in diastole, then the perfusate was prepared by mixing equal proportions of half-saturated solutions. When this was effected, the combination produced a gradual decrease in both systole and diastole. With arresting concentrations, the writing point stopped in a midway position. In balanced mixtures, then, each drug exhibited its individual effect.

In the case of rabbits and cats, mixtures of indol and skatol produced simple summation. This was to be expected in view of similar actions individually.

5. *Indol and Skatol on Other Nonvoluntary Muscle.*—In studying the effects of indol and skatol on other nonvoluntary muscle, the following organs were examined, suspended in Tyrode's solution at 37° C.: Intestine of the rabbit and the rat, uterus of the rabbit, and the vagina of the rabbit. Indol and skatol produced identical effects, depressing the musculature of all. One-eighth saturated solutions produced slowing of the rhythm without appreciable change in tone or amplitude; one-fourth saturated, decrease in both rate and amplitude; one-half saturated, depressed tone, rate, and amplitude; while saturated, a sharp drop in tone and a decrease in amplitude and rate progressing to quiescence. Removal of the drug was followed by almost, but never complete, recovery of all the properties of the tissue.

DISCUSSION

It has been shown that indol and skatol are cardiac depressants, their effects being almost identical qualitatively. Indol appeared to be more rapid and more potent in its effects, but these differences are no doubt due to its greater solubility and diffusibility, though the methyl group of skatol may have been a factor.

Both drugs act independently of the nervous apparatus and hence must be direct muscular depressants. This view is supported by the observation that they depress nonvoluntary muscle in general as is evinced in the case of the gastrointestinal tract and the reproductive organs.

We can offer no explanation of the differences shown by indol and skatol on the turtle's heart: i.e., the decreased relaxation with indol and the decreased contraction with skatol. It was the usual phenomenon during the

early fall months but was inconstantly exhibited in the middle of the winter. Apparently it depended on the state of nutrition of the animal, those of the autumn being freshly caught while the winter ones had been kept under artificial conditions for about two months. This will be investigated further when material becomes available.

Other species did not exhibit definite qualitative differences as regards the two drugs, nor did other tissues. It may be noted here that isatin,[6] which has the same constitution as indol except for the replacement of two hydrogen atoms by oxygen, depressed all the functions of smooth muscle and gave the same effects as indol and skatol on the warm-blooded hearts, but decreased both systole and diastole in the cold-blooded like a balanced mixture of indol and skatol, the two drugs with which we are dealing in this paper.

The literature on indol and skatol from the experimental standpoint was reviewed above. The several observers cited noted circulatory depression but were not explicit as to the part played by the heart. We have shown them to be direct cardiac depressants.

SUMMARY

1. Indol and skatol decrease the amplitude and rate and even arrest the hearts of frogs, turtles, rabbits, and cats.

2. Indol and skatol affect the hearts of cold-blooded animals more actively than those of warm.

3. Indol is more effective than skatol and its latent period is shorter.

4. Indol and skatol do not give nice quantitative results—on one animal a one-fourth saturated solution may produce as great a response as a one-half saturated on another of the same species.

5. Indol and skatol become more effective on repetition, low concentrations produce (but tardily) the same effects as high, and withdrawal is not followed by complete recovery.

6. Indol and skatol are antagonized by epinephrine but not by atropine.

7. Indol and skatol differ qualitatively on the turtle's heart in that the former usually decreases relaxation and arrests in systole, while the latter usually decreases contractility and arrests in diastole.

8. Indol and skatol in sequence are antagonistic; but, mixed in balanced proportions, each exhibits its peculiar effect, both systole and diastole being decreased.

CONCLUSIONS

1. Indol and skatol are cardiac poisons.

2. They are cumulative in their action.

3. They act directly on the muscle substance.

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LABORATORY METHODS

A UNIVERSAL ARTIFICIAL RESPIRATION AND CLOSED ANESTHESIA MACHINE*

By D. E. Jackson, M.D., Cincinnati, Ohio

THE device here shown can be used both for artificial respiration and for anesthesia by the closed method. The machine will give either positive or negative pressure and either of these can be either intermittently interrupted or used as a constant pressure. There are three rates of interruptions (by a lever gate valve) which give a good range for intermittently inflating the lungs of the usual laboratory animals. The volume of air discharged at each interruption by the valve can be varied from zero up to a volume sufficient perhaps for a horse. And when constant pressure is used it can be varied in the same way.

By reference to the illustration it may be seen that the machine is constructed about a heavy iron framework made up of four heavy angle iron posts (1) which are curved outward at the bottom and thus serve as a base on which the machine rests. The four posts are firmly held together by two iron plates (2 and 3) and by an iron frame (4) at the bottom. A very accurately made (air tight) Crowell rotary air pump (5) rests on the plate (3) and serves to circulate the air (or anesthetic substances) through the closed system of tubes and reservoirs. A strong motor (26) turns the pump by means of the belt (25). A reversing switch is located just behind the motor, so that if the motor is turned forward the outlet (22) will deliver positive air pressure but if the motor be reversed this outlet will register negative pressure which may be used for aspirating the chest (for lung tracings, etc.) or for suction filtration, etc.

An interrupting valve (21) is located just behind the outlet tube (22). This interrupting valve is operated by a special mechanism driven by the cone pulley (23) which is fastened on the end of the pump spindle. This pulley has three grooves for the round belt (24) which drives the interrupting mechanism (not shown in the illustration).

From the positive side of the pump the air is driven through pipe (12) down into the large tubular reservoir (14) from which the compressed air escapes through tube (13) which leads to two valves, the first being the wheel valve (27) (which when opened will allow air to escape under constant pressure for blast lamps, etc.) and the second the four-way valve (18). This four-way valve is operated by means of the handle (19). When the handle is turned as shown in the illustration the compressed air passes from tube (13)

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upwards, then across and downwards through the four-way valve and by way of tube (15) to the bottom of the canister (16). The canister contains soda lime. Or, if desired a second canister holding a saturated solution of sodium hydrate and carrying a special water trap and water gauge may be substituted in place of the canister (16), which is held on by means of the screw latches (28—28). There are some advantages in favor of using the solution but there are other advantages in favor of the dry lumps of soda lime.

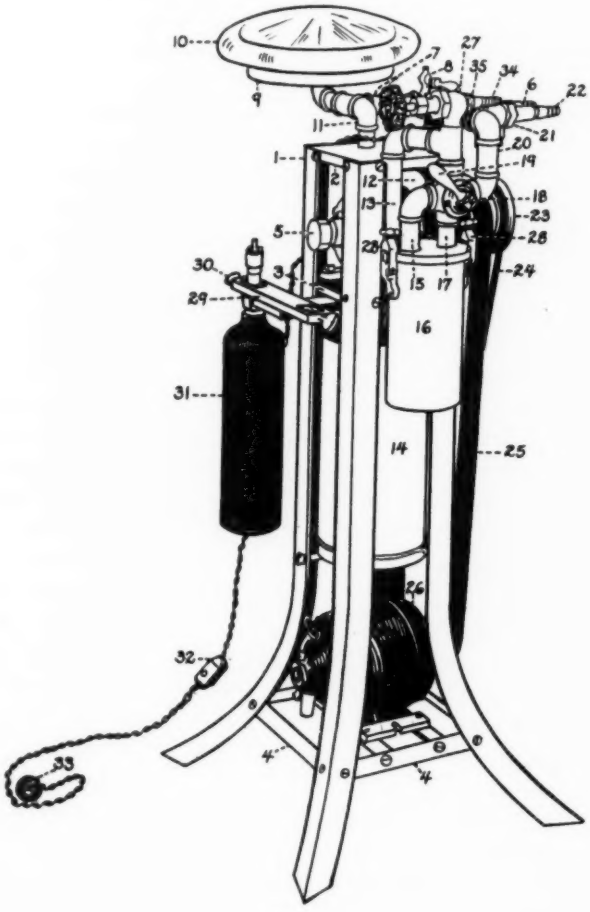

Fig. 1.

From the canister (16) the air (minus the carbon dioxide if the closed system is being used) passes out through tube (17) and again through the four-way valve (18) into the tube (20) which leads to the interrupting valve (21) and thence through the outlet (22) to the tracheal cannula. By turning the handle (19) of the four-way valve forward the canister is cut out of the system and the air passes directly from the reservoir (14) out through the outlet (22). In this way one can either filter out the CO_2 or allow it to accumulate in the system as may frequently be desired in experimental work.

Air enters the machine through the inlet shown at (6). This inlet tube

bends around, opposite and behind the wheel valve (27), and is continued as tube (7) which leads into the tube (11). This tube (11) connects below with the negative side of the pump (5) and above, by means of a tapered slip joint, with a large shallow pan (9) over the rim of which is stretched a bath cap (10). If desired the pan can be removed (at the slip joint) and a special L-tube, over which an ordinary anesthesia bag can be fastened, may be slipped in its place and used as the flexible reservoir in place of the (cheaper) bath cap. In either case the flexible reservoir serves to accommodate the breathing of the animal when the closed system is used. If the machine is used only for artificial respiration with air, or for blast lamps, negative filtration, etc., then neither the bath cap (nor anesthesia bag) nor canister (16) need be used. If the closed system is being used and too much gas (oxygen, ethylene, etc.) is run into the machine the excess may be quickly emptied out while the pump is running by slightly opening the wheel valve (27) for a few seconds.

Just behind the wheel valve (27) a small inlet air cock (8) passes into the inlet tube (6 * * * * 7). Through this air cock ethyl chloride, ether, chloroform, etc., or oxygen, acetylene, etc., from a separate cylinder may be injected at any time.

Between the course of the outlet tubes (20 * * * 21) and the course of the inlet tubes (6 * * * * 7) there is a connecting tube (35) in which is placed a by-pass valve. The handle of this valve (not numbered) is shown just above the wheel valve (27). When the by-pass valve is closed all the positive air pressure passes out through the outlet (22). But if the by-pass valve be wide open all the positive air pressure will pass through the connecting tube directly over into the (suction) inlet tube (6 * * * 7) and thence back into the pump. But if the by-pass valve is half open then half the positive pressure will pass out at the outlet (22) and half will pass back into the inlet side (negative pressure) of the system. By varying the amount of air passing through the by-pass any amount (volume) of air desired may be blown out at the outlet (22). Thus exactly the necessary amount of lung inflation can be secured. The by-pass valve acts in the same way if the motor be reversed and suction through the tube (22) be employed (for aspirating the chest in making lung tracings, etc.).

A set screw (30) serves to hold a cylinder (31) of oxygen, ethylene, nitrous oxide, propylene, etc., in the double yoke bar (29) which is supported from the plate (3). A small drilled hole and tubing carry the gases from the cylinders into the large reservoir (14).

Electric current for the motor (110 volts alternating or 110 volts direct—the proper ¼ horse power motor must be supplied for each current) is obtained through an extension cord shown at the left in the figure. The plug (33) attaches to a lamp socket or other outlet and the current is controlled by the switch (32).

The tubing used in the machine is brass and all joints are soldered. The supporting plate to which the canister (16) is attached carries a rubber gasket on its lower surface and the upper rim of the canister is drawn up tightly against the gasket and plate by means of the set screws in the latches (28—28). Thus the closed system can be made air tight throughout.

If the machine is used only for inflating the lungs with air as in ordinary artificial respiration, then a rubber tube $\frac{3}{8}$ inch in diameter and two or three feet long is attached to the outlet (22) and carried over to the animal where it is attached to the tracheal cannula. The tracheal cannula is best made in the form of a T-tube and the rubber tube leading from the respiration machine is attached to the side tube of the cannula. The straight end of the cannula (away from the animal) carries a short piece of rubber tubing on which is placed an adjustable screw clamp. By means of this clamp any excess of air which the machine would blow into the lungs may be allowed to escape and the degree of lung inflation can be controlled exactly as desired.

If, however, it is desired to use the closed system (with ethylene, etc.) a slight modification of technic is necessary. In this case two rubber tubes lead from the machine (outlet 22 and inlet 6) to the animal. If the animal is not to be operated upon and the trachea is not to be opened then a metal mask carrying a heavy perforated rubber membrane (through which the animal's nose is thrust) is strapped tightly to the animal's head. A tubulature in the mask carries a large, perforated cork. Through the hole in this cork is thrust one end of a special tracheal cannula. This cannula is of the T-tube variety but has two side tubes instead of one. The rubber tubes from the respiration machine are attached to the two side tubes of the cannula. Then one end of the cannula is passed through the cork in the mask and a short piece of rubber tubing carrying a screw clamp is used to close the distal end of the cannula. The mask is then placed on the animal and the machine started. The interrupting valve (21) need not be used in this case and is thrown out of action. The machine now merely circulates the anesthetic mixture round and round, the CO_2 being filtered out as desired.

But if one desires he may quite easily open the chest and still use the closed system, employing ethylene, etc., as the anesthetic. This is done by a slight modification of the above technic. The double-side-outlet tracheal cannula is used. But in this case a small screw clamp is placed on the end of the negative rubber tube near to the side tube of the tracheal cannula and the distal end of the cannula is closed tightly. If the cannula is now tied tightly into the trachea (instead of being pushed through the hole in a cork as in the above technic) it will be seen that air will be blown from the machine through the positive tube to the tracheal cannula and thence into the lungs. But if the (negative) tube leading from the tracheal cannula back to the inlet of the machine is of the same size as the tube which carries the current of air from the machine into the cannula, then most of the air will simply circulate around through the cannula and go directly back through the inlet tube into the machine without having inflated the lungs at all. This difficulty is overcome by simply partly closing the screw clamp on the negative rubber tube very close up to the side-tube of the tracheal cannula. This makes it more difficult for the air to pass out of the cannula back to the machine, and since an excess of air is blown by the machine into the cannula at each intermittent discharge it is seen that the lungs will be inflated by this excess which, just as soon as the machine ceases to discharge air from the positive tube,

will again pass back (a little more slowly) through the constricted part of the tube (where the screw clamp is partly closed) and on into the machine. By properly adjusting the screw clamp the degree of lung inflation can be controlled perfectly, either with the chest opened or closed.

If desired an ordinary etherizing bottle may be attached to the inlet tube (6) and the ether vapor thus drawn into the machine can be used to maintain the anesthesia.

The machine has been designed to cover a very wide range of experimental work, and if desired a recording spirometer can be attached (at the slip joint) in place of the bath cap or anesthesia bag. The machine has a capacity which is more than ample for experimental work on man, and I have no doubt but that it could be used for this purpose although I have not as yet carried out any such experiments.

For many years I have used in one device or another all of the principles involved in this machine. At the October meeting, 1923, of the anesthetists in Chicago I described a machine (with lantern slides) which did exactly the work which this machine does. And the earlier machine I also demonstrated in experiments carried out in June, 1924, in Prof. McGuigan's laboratory in Chicago. Recently[1] Prof. Starling in England has described an artificial respiration machine which involves some of the principles used in this machine.

The machine as here described may be obtained from the Max Wocher & Son Co., Cincinnati, Ohio.

REFERENCE

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GASTRIC MOVEMENTS IN THE PIGEON WITH ECONOMY OF ANIMAL MATERIAL. COMPARATIVE STUDIES V*

By T. L. Patterson, A.M., M.S., Ph.D., Detroit, Mich.

A FEW years ago I[1] published a method for studying the movements of the empty and filled stomach of the bullfrog which had given a certain degree of satisfaction in the hands of laboratory students. As pointed out in this article the methods as applied to the study of the movements of the gastrointestinal tract both in hunger and digestion have been carried out largely on man and the higher laboratory animals. In larger animals such as dogs with gastric fistulae a study of peristalsis can only be made after a period of intensive training and under the quietest surroundings.

Rogers[2] has shown that the contractions of the empty crop of the pigeon may be studied either by the balloon method or by direct observation. In the normal bird these contractions are not easy to demonstrate except by the balloon method, since the hungry animal in a cage is in a state of restless excitement, in which it can be seen only that the crop is empty. If such a bird be quieted, however, in a partially darkened cage with the observer sitting quietly at the side, the contractions of the empty crop can be seen without any form of registering apparatus. This demonstrates that the presence of the balloon in the crop does not necessarily act as the stimulus to the contractions and overcomes the objection of some to the balloon method for this type of work. The failure of Rossi[3] and Doyon[4] to control the inhibitory influences is probably one reason for these investigators claiming that the empty crop is quiescent. The placing of blinds over the bird's eyes usually has the same quieting influence as darkening the cage. Under these conditions with the bird quiet, one is able to observe at intervals one or more deep peristaltic waves running over the entire crop and this is frequently the precursor of the bird becoming restless. At other times, instead of the periodic contractions the entire crop may be so constricted as to nearly obliterate its lumen. This indicates that there is a relation between tonus and distension as described by Cannon[5] for the mammalian stomach, since peristaltic contractions will not appear on such a constricted organ. Between this high degree of constriction and that of a partially relaxed crop over which run deep peristaltic waves, there may be found all intermediate gradations in the same bird at different times.

The preceding facts which with patience can be observed in the normal bird are more readily demonstrated in the decerebrate animal, for here the inhibitory influences are at a minimum. The behavior of the crop of the operated bird is practically identical with that of the normal, with the exception, that the gastric activities are no longer related to distant influences.

*From the Physiological Laboratory of the Detroit College of Medicine and Surgery.
Read before the Fortieth Annual Meeting of the Iowa Academy of Science at Cedar Rapids, Iowa, April 30 and May 1, 1926.

The procedure involves etherization of the bird and surgical removal of all the forebrain anterior to the thalamus. Elaborate aseptic precautions in pigeons are unnecessary. The chief difficulty is in removing all of the forebrain without causing injury to the thalamus and cerebellum or injuring the cerebral circulation. The technic employed after etherization, consists of clipping the feathers from the top of the head and exposing the cranium by a longitudinal incision through the skin. With a scalpel, a small opening is drilled through the bone on either side overlying the cerebral hemispheres of sufficient size to admit the point of a scissors blade and the bone is then carefully removed, with the exception of that portion directly over the median sinus. The dura should not be torn during this process. Then with fine pointed scissors an incision is made through the dura over both hemispheres. All this can be done practically without bleeding. A probe with the point curved to fit the posterior border of the cerebrum is then introduced under the dura and the brain substance removed, one side at a time, while the dura

Fig. 1.—Dorsal aspect of head of pigeon after bone excavation and removal of cerebral hemispheres. Note the size and position of the bone openings, the bridge of bone in center protecting the median sinus and the mid and hind brain showing within the posterior portion of the cavity.

is left intact. Hemorrhage is controlled by gently packing with cotton. When the bleeding subsides, the cotton is removed, leaving the cavity empty and the skin is sutured over the bridge of bone which protects the median sinus (Fig. 1). A thin coat of collodium over the incision completes the procedure.

A fistula is now made in the crop directly following the decerebration and before the animal recovers from the anesthetic. The procedure consists of clipping off the feathers close to the skin from the lower end of the neck and the upper part of the breast. A small incision is made through the skin and the muscularis of the crop. This incision forms the fistula into which is inserted a piece of soft rubber tubing about two inches in length and one-quarter inch in diameter, the tubing being slightly larger than the opening, thus putting the tissue around the fistula on the stretch and making secure the tube. No sutures are required. The crop end of the tube is cut obliquely while

the exterior end is closed with a cork which is made secure by a safety pin and this also prevents the tube from being drawn into the crop. If the tube is removed the fistula will close in two or three days and the animal is none the worse for the operation. Direct observation shows that such fistulae do not modify, or only vary slightly the normal movements of the crop. In twenty-four hours the fistula tube may be removed and a rubber balloon introduced into the crop and connected with a water manometer for graphic registration of the movements (Fig. 2). A balloon about 4 by 4 cm. of the condom type is used and is tied with a silk thread onto the end of a flexible rubber tube about 4 mm. outside diameter which contains a small metal or glass cylinder about 8 mm. in length of such a size as to exactly fit into the lumen of the tube.

The gastric apparatus of the pigeon is anatomically divided into three parts: the crop, usually considered as a simple dilatation of the esophagus

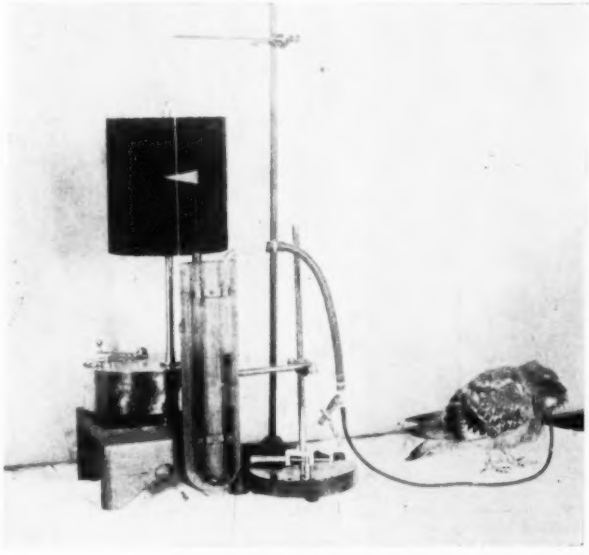

Fig. 2.—Typical decerebrate pigeon, feathers raised, head drawn in to shoulders, eyes closed, with balloon in crop connected with registering apparatus.

and similar in structure to it,[6] the proventriculus or glandular stomach, and the gizzard or muscular stomach. All parts of this apparatus exhibit motility and according to Kato[7] the pressure exerted by the contractions of the gizzard during hunger are greater than those occurring after feeding. These contractions may be obtained by simply pushing the balloon into the gizzard through a fistula made in the midline of the crop. From the standpoint of comparative anatomy, the crop of the bird may also be considered to correspond to the cardia of the stomach of higher animals.

The hunger contractions obtained from the empty crop like those obtained from the gizzard in fasting are more vigorous than the digestive peristalsis after feeding. The hunger contractions of the empty crop exhibit a definite periodicity characteristic of the behavior of the empty stomach of higher animals (Fig. 4). Sometimes the contractions are rapidly and con-

tinuously repeated for several hours, but they usually occur in groups periodically. On the contrary, the movements of the filled crop are of less amplitude, more irregular and less indicative of a definite periodicity (Fig. 5). After feeding the hunger contractions are usually entirely absent for thirty to forty-five minutes. Then at short intervals, irregular contractions begin to occur which after an hour or two gradually increase in frequency and vigor, first appearing in groups of three or four waves and then after five or six hours in groups of six to twelve or more, separated by intervals in which the crop is comparatively at rest. It requires from ten to twenty seconds for each peristaltic wave of the empty crop to complete its cycle, whereas the more rapidly repeated contractions of the lower part of the crop occur at the rate of eight to ten per minute. If the bird sets up a shrugging side-

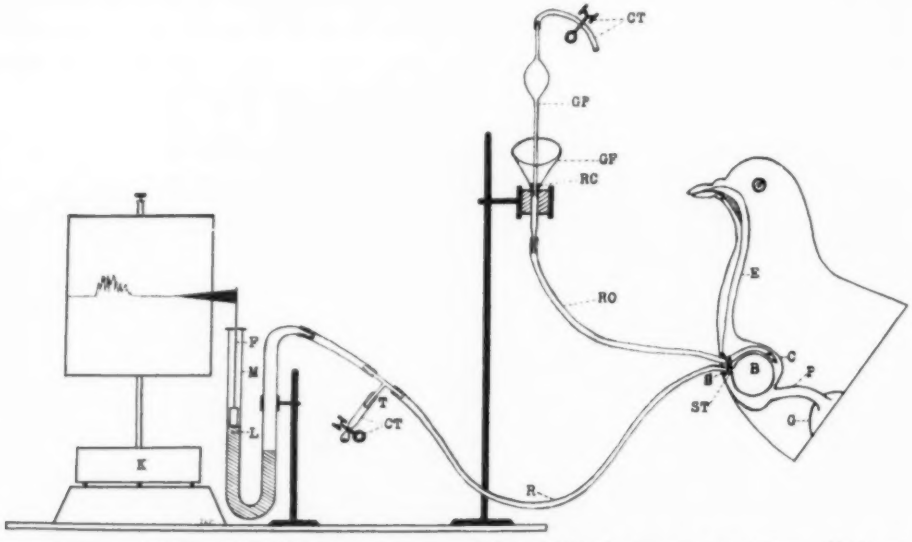

Fig. 3.—Diagram showing method of recording peristalsis of crop of pigeon. *K*, kymograph. *F*, glass float with recording flag. *M*, manometer. *L*, manometer liquid (water). *T*, glass T-tube for inflation of balloon. *R*, rubber tube connecting balloon with manometer. *S*, crop fistula. *B*, rubber balloon in crop. *E*, esophagus. *C*, crop. *P*, proventriculus. *G*, gizzard. *CT*, clamp and rubber tube. *GP*, glass pipette. *RC*, rubber cuff on end of pipette. *GF*, glass funnel. *RO*, rubber tube with open end in crop. *ST*, silk thread holding tubes together.

wise movement it indicates overdistension of the balloon or that the balloon is too large and it then becomes a source of irritation. Sometimes a similar condition results from overdistension of the crop with food.

For studying the influence of inhibitory substances (liquids) on the movements of the empty crop or the stomach of other animals, a second rubber tube is attached to the basal portion of the balloon tube with a silk thread with its open end extending to the midportion of the balloon. The other end of the tube is connected to the stem of a small glass funnel which has been heated and drawn out to fit the tube. Within the funnel, a pipette with a tapering end fitted with a rubber cuff fits tightly into the opening of the funnel stem. A rubber tube and clamp completes the pipette, so that any desired fluid may be retained therein until the desired moment, when by releasing the clamp it may be permitted to flow slowly through the tube and

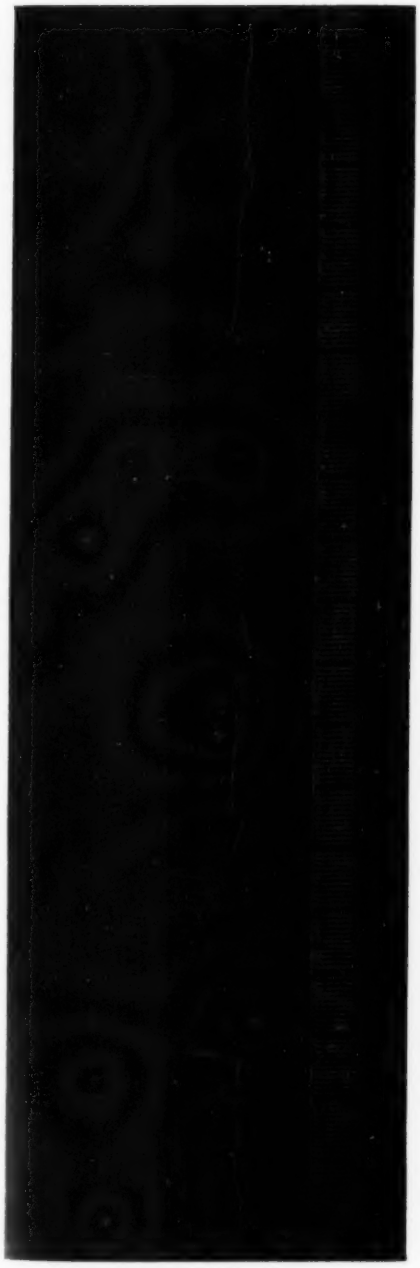

Fig. 4.—Decerebrate pigeon. *A*, peristatic contractions of crop twenty-four hours after feeding, crop empty. Note the periodicity characteristic of the behavior of the empty stomach of higher animals (hunger peristalsis). *B*, time in five second intervals. Water manometer.

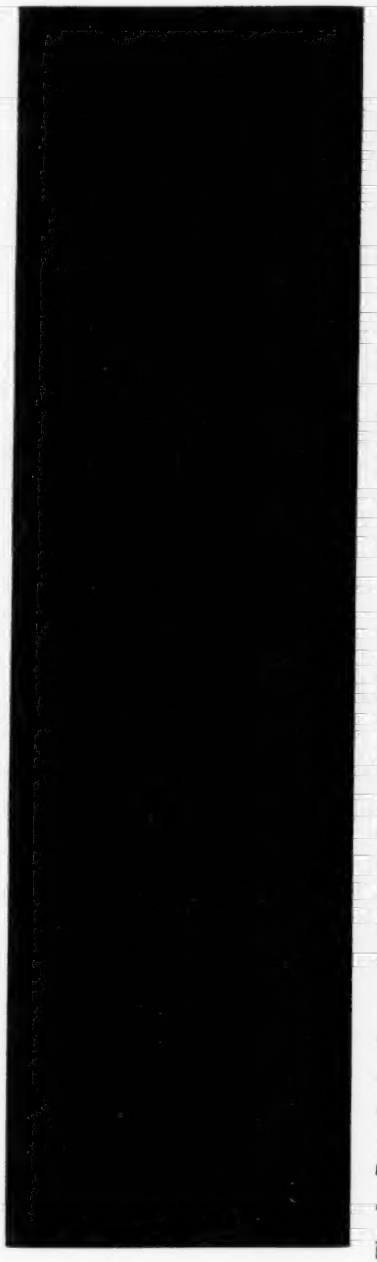

Fig. 5.—Decerebrate pigeon. *A*, digestive peristalsis of crop thirty to forty-five minutes after feeding. Food in crop. *B*, time in five second intervals. Water manometer.

directly into the crop (Fig. 3). In the case of water there appears to be some quantitative relation between the volume of fluid introduced and the relaxation of tonus. Thus 2 to 4 c.c. of water does not always produce this inhibitory action, while 8 to 12 c.c. is effective (Fig. 6). More recently it has been found that very small amounts of food given to monkeys do not lead usually to gastric inhibition, while larger quantities prove effective.[8] In

cases where a study of inhibition is desired a larger fistula should be made in the crop and a rubber tube one-half inch in diameter inserted.

It is therefore possible to utilize to advantage the decerebrate pigeon for a study of peristalsis for removal of the cerebral hemispheres does not materially affect the peristaltic movements of the crop. This animal is even more suitable for use in the general student laboratory for such a study than the bullfrog, since decerebration transforms the nervous active bird into a stupid, lethargic creature which reacts only when stimulated. Hence, the bird in addition to exhibiting the classical effects of decerebration may be satisfactorily utilized even under the disturbing influences of the student

Fig. 6.—Decerebrate pigeon. *A*, hunger contractions of crop thirty-two hours after feeding, crop empty. *B*, signal magnet. At *X*, 8 c.c. water at room temperature was introduced directly into crop. Note the abrupt termination of the period of hunger activity, inhibition. Water manometer. (Tracing reduced about one-half.)

laboratory for a study of peristalsis. Furthermore, bullfrogs are difficult to obtain during certain seasons of the year while pigeons are more readily obtainable and this coupled with the combined studies of decerebration and gastric peristalsis on one and the same animal leads to an economy of animal material and a financial saving.

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A SIMPLIFIED METHOD FOR THE PREPARATION OF SPECIAL LIQUID BLOOD MEDIA BY FILTRATION*

By A. J. Salle, Ph.D., Berkeley, Calif.

During the past two years I have used large quantities of hemoglobin media for metabolism experiments and have found it exceedingly difficult to obtain sterile filtrates. The trouble does not rest so much in obtaining a sterile filtrate in the receptacle, but in the manipulations of transferring it from the flask to sterile containers. When a quantity of about 200 c.c. of filtered medium is distributed the environment must be absolutely dust free, or otherwise, contaminations are the rule.

The preparation of hemoglobin mediums is always a time consuming process. It was usually handled as follows: An animal was exsanguinated, the blood laked and the hemoglobin solution mixed with the medium. The whole was centrifuged before passing it through a sterile Mandler filter. A contamination of the filtered medium always meant a considerable loss of time and material.

In order to overcome the disadvantages of the usual methods of filtration the following method has been adopted. Two Pyrex filter flasks, *A* and *B*, of 250 c.c. capacity each, are connected to a T-tube *C* by means of two pieces of pressure tubing of one-eighth inch bore and one-eighth inch wall. This T-tube has an outer diameter of one-fourth inch and a slight enlargement near the opening of each arm. The two lower enlargements are to hold the rubber tubing tightly while the upper one is to hold a tight packing of cotton. Both flasks take a No. 7 rubber stopper.

A glass tube *F*, three-eighths of an inch outside diameter, projects through stopper *D* containing a plug of cotton and gauze in the upper end. Stopper *D* is now inserted into the flask and a layer of cotton wrapped around the mouth to prevent contamination. This cotton is covered with a piece of paper and fastened by means of a rubber band. A piece of paper is placed over the opening of tube *F*. This is also fastened with a rubber band. The preparation of flask *B* is thus complete.

Stopper *E* contains two glass tubes, the larger one one-fourth inch outside diameter and the smaller one three-sixteenths inch outside diameter. The smaller tube contains an enlargement at the upper end to hold a tight packing of cotton. A rubber tube *L* is slipped over this enlargement; the open end is closed by means of a piece of solid glass tubing. Stopper *E* is now inserted into the flask and covered with cotton and paper similar to stopper *D*. A piece of pressure tubing *G* projects from the upper end of the larger tube. The opening *H* is also covered with paper and fastened with a rubber band.

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This completes the preparation of flask *A*. Both are sterilized in the autoclave, after which they are ready for use.

The filter candles are wrapped in paper and sterilized as customary. The wrapping around a sterile candle *I* and the paper covering *H* are both removed, the openings flamed and the candle inserted into the pressure tubing. The mantle of the candle is fastened to a ringstand.

When the setup is complete the medium is placed on the filter and the pump connected with the apparatus through tube opening *C*. A screw clamp is placed at *K* in order that no air will be drawn through at *F*. After the stopper *E* is forced tightly into the neck of the flask the pump is turned on. The filtered medium collects in *A*.

When the filtration is complete, the pump is shut off, the screw clamp at *K* is removed and placed at *G*. The candle is then disconnected from the

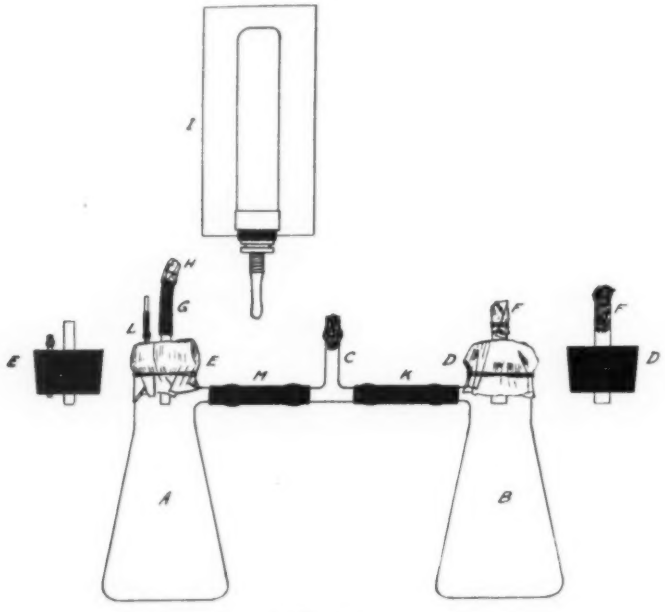

Fig. 1.

flask. The glass rod and rubber tube *L* are removed to let air into the flask. By raising flask *A* one-half of the medium is poured into flask *B*. Screw clamps are placed at *M* and *K*, after which the T-tube connecting the two flasks is removed. Flask *A* serves as a control while flask *B* is inoculated through the tube at *F*.

With such an arrangement only two sources for contamination must be considered: (1) The manipulations while connecting the candle to the pressure tube and (2) while making the inoculations through *F*. Since these openings are very small the chances for contaminations are slight. Over fifty samples of media have been filtered by means of this apparatus with only one contamination, while with the ordinary method they were the rule rather than the exception.

AN IMPROVISED METAL CANNULA*

By R. R. Durant, M.Sc., Columbus, Ohio

THE cannula is an instrument of general and varied use in physiologic experimentation. A large number, varying more or less in style and material are in use. Each has its good and bad qualities, which fact has led the writer to develop the use of the instrument described here. To be satisfactory a cannula should be cheap and durable, easy to handle and interfere as little as possible with the natural phenomena being observed. Credit for the original suggestion is due Dr. E. C. Albritton formerly a Fellow in this department.

The instrument is made from a Luer hypodermic needle of desirable size and length, having the end smoothed and a small knob of solder placed on one side (Fig. 1).

In this laboratory, cannulae of this type are used wherever permanent connection with a vessel is desired, the trachea of course excepted.

For recording of blood pressure by the direct method a needle of approximately 1 cm. in length is used. After being tied into the artery it is connected with the manometer by means of a tapered adapter (Fig. 2), similar to that at the outlet of a syringe. A slight twist secures it against coming off even when subjected to the greatest arterial pressure. A twist is sufficient for removal during or at close of the experiment. The size required varies with the size of the animal. For large dogs a gauge 12 needle has a lumen of sufficient size that the actual blood pressure will not be reduced. A gauge 15 needle accommodates the artery of an average sized cat or rabbit. For small animals, rat, guinea pig or kitten the smaller needles—gauge 24-18—will suffice. Arterial cannulae of this type have a wall of minimum thickness and consequently a relatively large lumen. The danger of clotting in the cannula is correspondingly reduced. Using a gauge 15 needle a continuous blood pressure

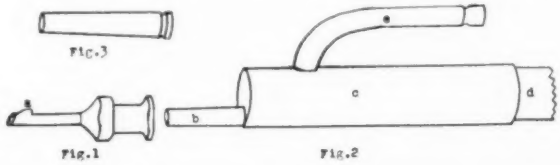

Fig. 1.—Hypodermic needle shortened according to need; tip beveled and smoothed and a drop of solder (*a*), placed near tip.

Fig. 2.—Connection for recording blood pressure. (*b*) Adapter, having a taper of 1.5° to fit the cannula, turned from brass tubing (not nickeled), and soldered to (*c*) reservoir for mixing anticoagulant with the blood; made of brass tubing 10 mm. in diameter and approximately 5 cm. long. (*d*) Block tin pipe having inside diameter, 3/16 in. and 3/64 in. wall, approximately one foot long, supported by means of a burette clamp on the stand bearing the manometer and signal magnets. (*e*) Brass tube 6 mm. in diameter to receive the rubber tube from the pressure bottle. (*c*) and (*e*) may be nickeled.

Fig. 3.—Adapter similar to that in Fig. 2, (*b*), but slightly longer and grooved near the end as illustrated for securing the rubber tube connecting with pressure bottle.

*From the Department of Physiology, College of Medicine, Ohio State University.
Received for publication, January 19, 1917.

record can be made for a maximum allowable period of time (one to three hours) without clotting. Records lasting ten to twenty minutes can be obtained without clotting using a gauge 20 needle. It is obvious that the use of such a cannula eliminates the necessity for various sizes of tubing, etc. The blood pressures of an 80 gm. rat and 15 kg. dog have been recorded with the same apparatus by simply changing the cannulae. That the use of the smaller cannulae gives fairly accurate results is shown by simultaneously recording the pressure in two paired arteries equidistant from the heart, using gauge 20 and 15 needles respectively. The pressure in the first artery is found to be only 5 mm. Hg. lower than that in the second.

Cannulae of the same type can be used for intravenous injection or for cannulating ducts. A syringe can be used for the reservoir and source of pressure or a hub (Fig. 3) may make connection with a pressure bottle. A gauge 22 needle makes an easily applied cannula for use in recording urine flow from the ureter, even in very small animals, and for cannulating the ducts of submaxillary and pancreatic glands. Cannulae of this type have the following qualities for which it is believed their use will be found to relieve some of the difficulties of laboratory work.

1. Expense. The cost is hardly more than that of the needle.

2. Durability. Ordinary use is never strenuous enough to break such cannulae.

3. Ease of Use. The cumbersomeness of glass T cannulae is eliminated; slipping out of the vessel is nearly impossible; small and delicate vessels can be cannulated with little danger of tearing; connection with other apparatus is simple.

4. The relatively large lumen affords free passage of fluids and therefore reduces error such as is unavoidable in the use of small glass cannulae.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

CLINICAL AND EXPERIMENTAL

MENINGITIS: The Chemotherapy and Serum Therapy of Pneumococcus and Streptococcus Meningitis, Kolmer, J. A. Arch. Otolaryngology, June, 1926, iii, 481.

Polyvalent antistreptococcus serum administered by intramuscular, intravenous, intracisternal and intraventricular injection has failed to influence appreciably the cause and mortality of experimental hemolytic streptococcus meningitis of dogs.

Type I antipneumococcus serum administered by intramuscular, intravenous, intracisternal and intraventricular injection has failed to influence appreciably the course and mortality of experimental type I pneumococcus meningitis of dogs.

The intravenous, intraventricular and intracisternal injection of antipneumococcus antibody solution has exerted a slight degree of curative activity in the treatment of pneumococcus meningitis.

Lavage of one or both of the lateral ventricles to the cisterna magna has proved effective in the treatment of severe experiment streptococcus and pneumococcus meningitis of dogs. This may be combined with the administration of antibody solution by subthecal injection in the treatment of pneumococcus meningitis, or by the injection of gentian violet in the treatment of streptococcus meningitis.

NEOPLASMS: The Etiology of Malignant Neoplasms, London, J., McCormack, J. M., and Howard, N. J. Canadian Med. Assn. Jour., May, 1926, xvi, No. 5, p. 523.

The authors believe the cause of neoplasms to be a Gram-positive pleomorphic organism which they have isolated from fresh human neoplastic tissues and from neoplasms in rats and chickens and from the blood of the hosts. In one of its phases this organism is filterable and invisible under the microscope.

It is admitted that infection does not usually take place unless the defences of the cell have been broken by some preceding condition which lowers resistance to infection.

The paper is a summary of previous publications and studies to date.

LEUCEMIA: Leucemia and the Central Nervous System, Fried, B. M. Arch. Path. and Lab. Med., July, 1926, ii, No. 1, p. 23.

A case of leucemic lymphadenosis is reported, and thirty cases of leucemic lymphadenosis and myeloadenosis from the literature, involving the central nervous system, are reviewed.

In the author's case a patient with a subacute leucemic lymphadenosis died of apoplexy, and at necropsy numerous lymphomas and hemorrhages were found in the brain. Grave degenerative changes were found in the interstitial, parenchymatous and mesenchymal elements of the brain, more pronounced in the vicinity of the extravasated blood and also around the lymphomas. These changes are not regarded as peculiar to leucemia, but are ascribed to circulatory disturbances in the brain due to hemorrhages and accumulated masses of lymphoid elements (similar lesions of a minor degree occur in primary and secondary malignancy of the brain).

In the thirty cases gathered from the literature, lesions of the nervous system have been reported in the hemispheres in twelve, in the cranial nerves in eight, and in the cord in eleven cases, in eight of which spinal degenerations were observed in the absence of lymphomas, in many respects resembling those observed in pernicious anemia.

Hemorrhages in leucemia are primarily due to vascular lesions produced by (1) stasis in the capillaries and "thrombosis" of the vessels by lymphoid cells, (2) the invasion of the vessel walls by the lymphoid cells with dissociation of the vascular coat and (3) the hypothetic "toxin" which circulates in leucemic blood.

The occurrence of lymphomas in organs or structures in which lymphoid tissue is normally absent (as in the brain) is due to the passage of the vascular wall by lymphoid cells, with the ultimate formation of large nodules which occasionally grow as autochthonous units. This phenomenon is defined as "colonization" by lymphoid elements and is to be distinguished by metastases in malignancy.

PERNICIOUS ANEMIA: Familial Pernicious Anemia, Dorst, S. E. Am. Jour. Med. Sc., August, 1926, clxxii, No. 2, p. 173.

The recent observation of a patient with pernicious anemia, five other members of whose family had died of it or had had the same disease, suggested an investigation of the other members of the family group, which disclosed the fact that four more had either marked hypochlorhydria or complete achlorhydria.

Dorst feels certain that if it can be shown that achlorhydria is a predominating characteristic in families, one or more members of which have pernicious anemia, then there would seem to be no question but that "congenital" achylia gastrica is undoubtedly the hereditary factor in cases of familial Addison's anemia, and that gastric analysis in the members of the immediate family of a patient with pernicious anemia would show evidence of achylia gastrica, of congenital or hereditary origin.

A detailed account is given of the cases mentioned above and Dorst concluded with these queries: Can the development of pernicious anemia be prevented by the administration of hydrochloric acid to patients who have an achlorhydria and a familial history of the disease? Is similar therapy of value in the early stages of the disease? These questions cannot be answered until our clinical cases have been followed for the next five or ten years, or without an exhaustive experimental investigation.

LABORATORY TECHNIC

TISSUE TECHNIC: A New Method of Mounting Fixed Frozen Sections, Campbell, L. D. Arch. Path. and Lab. Med., June, 1926, i, No. 6, p. 916.

It is easier to work over a black table. The steps are as follows:

1. Previously fixed tissues are cut with a freezing microtome in sections 7 to 12 microns thick, which are placed in a large glass dish containing water.

2. A section is lifted out with a glass rod with the section wrapped smoothly around the rod near its end, and held in 95 per cent alcohol for two or three seconds.

3. The section is returned to a large, deep staining dish of water (about 7 cm. deep), unrolling the section from the rod on top of the water. In most cases it will float on the surface and be perfectly smooth.

4. A glass slide is brought against one edge of the floating section, holding the slide at almost right angles to the surface, and is lifted out. The section will adhere to the slide. If it has not been held in alcohol sufficiently long, or if too much time elapses before mounting after returning to the water, it will sink beneath the surface and cannot be readily mounted. If a fold should occur, the slide should be held on the edge and dipped carefully up and down in the water (not immersing the entire section), and the fold will easily be removed.

5. The mount is completed as usual by dropping on 95 per cent alcohol to dehydrate. It is blotted with a smooth folded cloth, and very thin celloidin is dropped on the section, holding the slide almost perpendicular to the table.

When the celloidin begins to solidify it is to be stained as desired.

UREA IN SALIVA: A Quantitative Method for the Determination of the Combined Urea and Ammonia Nitrogen of Saliva, Schultz, F. W., and Ziegler, M. R. Am. Jour. Dis. Child., April, 1926, xxxi, 520.

Ten c.c. saliva collected without the use of a stimulant is immediately shaken for five minutes with 2 gm. of nitrogen-free kaolin and filtered. The kaolin is prepared in quantity from Merck's kaolin by washing twice with 2 per cent acetic acid, four or five times with distilled water, filtering and drying. If the filtrate is turbid, the process is repeated. To 1 c.c. of the saliva filtrate in a 75 c.c. pyrex test tube add 4 c.c. of distilled water, 2 drops of Folin's pyrophosphate buffer mixture and 1 c.c. of his urease solution. This is heated in a water-bath, from 40° to 55° C. for five minutes. The ammonia, including that formed from the urea, is removed by aeration after the addition to the digestion mixture of a little liquid petroleum and 2 c.c. of 10 per cent sodium hydroxide. It is collected in a test tube graduated at 25 c.c. and containing 2 c.c. of 0.05 normal hydrochloric acid diluted with about 10 c.c. of water. After fifteen minutes aeration, dilute the contents of the receiver to 20 c.c., add 2.5 c.c. of Nessler's solution, make up to 25 c.c. and compare in the colorimeter with a standard containing 0.3 mg. of nitrogen (in the form of ammonium sulphate) and 10 c.c. of Nessler's solution in a 100 c.c. flask.

Calculation:

$$\frac{\text{Reading of standard in mm.}}{\text{Reading of unknown in mm.}} \times 0.3 \times \frac{1}{4} \times \frac{100}{1} = \text{mg. of ammonia and urea N per 100 c.c. of saliva.}$$

Samples of blood and saliva were collected simultaneously. The determination of blood urea nitrogen was carried out according to the directions of Folin and Wu, using the aeration method.

AMEBIC INFECTION: Detection of Amebae in Cases of Chronic Systemic Amebic Infection, Albert, H. Am. Jour. Pub. Health, April, 1926.

Directions for the collection of specimens of feces to be examined for amebae:

1. Give patient eighteen 5-grain tablets of Glycotauro (bile salts) with instructions to take one tablet two hours after each meal for six days.

2. Give patient six small wide-mouth well-stoppered bottles with instructions to collect a small portion of feces (about the size of a small marble) each day for six successive days, beginning the day after starting on the bile salts.

3. Send specimens to the laboratory as soon as possible. If the laboratory cannot be reached within a day, pour a small amount (about one-fourth volume of feces) of 10 per cent formalin (one part of the commercial formalin to ten parts of water) over the feces.

The laboratory examination consists of a search chiefly for the cyst forms of the parasite, since these are, as a rule, very much more numerous than the motile vegetative forms. This is especially true of chronic amebiasis. In cases which present a rather definite clinical picture of amebiasis and cysts have not been found on repeated examination, it is advisable to make an examination for the vegetative forms also. Specimens to be examined for such should be kept at body temperature by means of a vacuum bottle and a warm stage.

Technic of Examination:

Two methods are used: (1) a direct smear of fresh fecal matter stained with a modified Donaldson's iodine-eosin, and (2) a fixed preparation stained with Haidenhain's iron-hematoxylin.

A drop of normal salt solution and one of iodine-eosin stain are placed close together on a slide but not touching. A round applicator stick or a toothpick is smeared with the feces, rolled in the drop of normal salt, then in the drop of iodine-eosin. A single cover slip is placed on both drops, half the material under it being stained and the other half unstained. Examine the unstained portion first for living flagellates and active amebae. In the stained

portion the protozoan cysts stand out as bright spherules against the pink background and soon become tinged with the iodine to varying tones of yellow, with the nuclei becoming clearly defined as the iodine penetrates. If glycogen is present in the cysts, it becomes light or dark brown in color.

Iodine-eosin stain:

a. Saturated aqueous solution eosin in normal salt ---------- 2 parts
b. Five per cent potassium iodide in normal salt solution saturated with iodine ---------- 1 part
c. Normal salt solution ---------- 2 parts

The proportion of iodine solution used may be modified to advantage by adding a slight excess of that given in the formula if the nuclei do not appear after a few moments' application of the stain. The stain should be made up each day from the stock ingredients.

2. For fixed preparations a smear is made on a slide which has been previously thoroughly cleaned in alcohol-ether and flamed. If the fecal material is too dry, moisten slightly with normal salt, make a thin smear with the applicator stick or the flat side of a toothpick or by using the edge of another slide or a cover slip, and immerse directly in fixing fluid *without allowing the slide to become dry.*

Fixing and Staining Methods Used:

1. Schaudinn's fluid (even if previously fixed by formalin) ---------- 5 min.
2. Seventy per cent alcohol tinged with Gram's iodine ---------- 5 min.
3. Seventy per cent alcohol ---------- 5 min.
4. Fifty per cent alcohol ---------- 5 min.
5. Tap water ---------- 2 min.
6. Two per cent iron alum-aqueous solution ---------- 5 min. to 12 hrs.
   Or 2 per cent iron alum-aqueous solution heated to 30° C. (never higher) ---------- 10 min.
7. Tap water—running ---------- 5 min.
8. Five-tenths per cent hematoxylin-aqueous solution (Haidenhain's) ---------- 12 to 18 hrs.
   Or 0.5 per cent hematoxylin-aqueous solution heated to 30° C. (never higher) ---------- 10 min. to 1 hr.
9. Tap water rinse
10. Differentiate in 1 per cent iron alum with careful watching under the microscope ---------- 3 to 30 min.
11. Wash in running water ---------- 10 min.
12. Fifty per cent alcohol ---------- 5 min.
13. Seventy per cent alcohol ---------- 5 min.
14. Ninety per cent alcohol ---------- 5 min.
15. One hundred per cent alcohol ---------- 5 min.
16. Xylol ---------- 5 min.
17. Mount in balsam—cover.

Schaudinn's fluid—two parts saturated aqueous $HgCl_2$ in normal salt; one part absolute or 95 per cent alcohol. Add 4 c.c. glacial acetic acid to 96 c.c. of the mixture on using.

If a quick method is desired, the same outline can be followed with a shortening of the time of application of the iron alum and the hematoxylin. The slide is taken from the water, flooded with or in alum by a pipette and held over a flame or placed upon a heated plate for about five minutes, or until it begins to steam. Wash in water and treat in the same way with the hematoxylin, continuing the application by heat until a metallic scum appears on the top of the fluid on the cover slide. Differentiate in iron alum. Care must be taken throughout the entire process to avoid drying of the smear. Use American hematoxylin, standardized white crystals only. Use only violet crystals of iron alum; reject yellowish powder.

COLLOIDAL GOLD: The Correction of Colloidal Gold Solutions as Applied to the Lange Reaction, Novich, N. Arch. Neurol. and Psychiatry, April, 1926, xv, No. 4, p. 471.

METHOD OF PROCEDURE

NUMBER OF TUBE	1	2	3	4	5	6	7	8	9	10	11	CONTROL
Twentieth normal sodium hydroxide or twentieth normal hydrochloric acid (as required) cubic centimeters	0.05	0.075	0.1	0.15	0.2	0.25	0.275	0.3	0.35	0.375	1.40	None
Colloidal gold solution (as prepared) cubic centimeters	5	5	5	5	5	5	5	5	5	5	5	5
1 per cent sodium chloride solution, cubic centimeters	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7

The series of tubes thus set up are set aside at room temperature, protected from light, and read at the end of one hour. The tube showing complete precipitation and containing the least amount of acid or alkali is taken as the correction point for the solution. The amount needed for the whole is calculated and added to the prepared solution. The control tube serves as a preliminary indicator and shows whether acid or alkali is needed for correction. It is not advisable to use an acid or alkali solution of higher normality, because it would affect the strength of the sodium chloride solution used as an indicator, an important factor.

It should be remembered that a colloidal gold solution at best, when tested with a clinically pathologic cerebrospinal fluid, does not precipitate in the first or second tubes, or high concentration, at once, but the higher dilutions (1:160 to 1:320) take precedence and precipitate almost immediately. This is probably due to the fact that the high concentration of a fluid that has a high protein content (globulins) brings about a condition of surface tension unfavorable for immediate precipitation. It should also be remembered that the method of titrations, as outlined, cannot be used for solutions grossly improper because of noncompliance with technical requirements.

Conclusions:

1. In the preparation of a good colloidal gold solution, certain technical difficulties, at best unavoidable, are frequently encountered. Many solutions are discarded as unfit, though prepared with great care.

2. The primary cause of unsuitable solutions is the reaction of the final product.

3. The old method of titration, using alizarin as indicator, is not entirely satisfactory. It does not visibly "indicate" because of the primary color of the solution under titration.

4. A method of titration using 1 per cent sodium chloride solution to the extent of 1.7 c.c. as an indicator is suggested. This salt is an electrolyte and a precipitant of colloidal solutions, and serves as an accurate and highly satisfactory indicator of the reaction state of a prepared colloidal gold solution.

SPIROCHETA PALLIDA: Experiments on the Purification of Cultures of Spirocheta Pallida by Chemical Methods, Weiss, D., and Weiss, C. Jour. Infect. Dis., April, 1926, xxxviii, No. 4, p. 281.

Experiments were undertaken to determine the selective inhibitory action of various germicidal substances on the growth of bacteria which may contaminate cultures of Spirocheta pallida.

The following chemicals are satisfactory (in the dilution and time of exposure stated) for the purpose of destroying B. coli as well as Staphylococcus aureus without affecting the viability of the reproductive power of Spirocheta pallida; Selenium oxychloride or tricresol (in a 1:100 dilution, to be used for one minute); trichloracetic acid (1:100 for fifteen minutes) or formaldehyde (1:20 for five minutes).

When it is desired to destroy staphylococci alone, a larger variety of chemicals may be employed: Gentian violet, acid fuchsin, mercurochrome, mercurophen, methylene blue, monarsone, neoarsphenamine, atoxyl, acid arsphenamine, antiformin, Lugol's solution (iodine), ethylhydrocuprein hydrochloride or neosilvol.

TUBERCULOSIS: Culture of Tubercles in the Diagnosis of Tuberculosis, Hohn, J. München. med. Wchnschr., April 19, 1926, lxxiii, 609.

To 10 c.c. of material to be cultured add 1 to 2 c.c. of sulphuric acid 10 per cent in a test tube and allow to stand thirty minutes. The tubes are shaken from time to time. Centrifuge for five minutes and inoculate the sediment on three to four egg tubes.

GONORRHEA: The Diagnosis of Gonorrhea by Culture, Gradwohl, R. B. H. Jour. Am. Med. Assn., July 24, 1926, lxxxvii, 242.

The following media has given good results in the author's hands in primary cultures.

Five hundred grams of ground lean beef is infused in 500 c.c. of distilled water and allowed to stand in the ice box over night. The following morning, 30 gm. of agar is dissolved by boiling in 500 c.c. of distilled water. The agar is allowed to cool to between 60° and 70° C., and the meat infusion is immediately mixed with the agar. This mixture is again heated until the meat is thoroughly coagulated. It is then filtered through glass wool packed in the stem of a glass funnel. This filtrate will appear slightly cloudy at this time. In the filtrate is dissolved 1 per cent peptone and 0.5 per cent sodium chloride.

The medium is adjusted to P_H 7.6 to 7.8, allowed to cool to 60° C. and the white of an egg thoroughly mixed in. It is next brought to a boil and again filtered through glass wool. If the flask and filtering funnel are placed in an Arnold sterilizer, the filtration is hastened and a crystal clear agar results.

To this medium is added 1 per cent chemically pure levulose (made from inulin), and sufficient 0.5 per cent aqueous bromcresol purple indicator to color the medium a rich purple. The whole is then autoclaved at five pounds pressure for forty-five minutes. When the medium has cooled to between 60° and 70° C. one part of sterile ascitic fluid, guinea pig serum or human serum is added to three parts of medium, and the plates are poured to a depth of about one-eighth inch. The plates may then be stored in the ice box until ready for use. In inoculating plates, best results will be obtained if straight line streaks are made with a platinum loop, thus preventing confluence of colonies.

In the cover of the Petri dish is inserted a piece of common blotting paper saturated with hydrogen peroxide. The plate is then inverted and incubated over night, and the next morning the suspicious colonies are fished and stained by Gram's method.

All colonies exhibiting a zone of yellow (showing acid production from levulose) are disregarded. All colonies showing an opaque whitish luster similar to Staphylococcus albus are disregarded. Typical colonies of Neisseria gonorrhea can be identified by the low power objective of the microscope, the 9x eyepiece or even by the naked eye after practice. The colonies in eighteen hours are about the size of a pin head, round translucent, finely granular, and with a pearly opalescence by transmitted light—the latter characteristic being always striking. The colonies also are mucoid, adhering to the medium, though emulsifying in water fairly easily. On staining, the individual organisms are moderately large, decolorize very quickly in 95 per cent alcohol, and exhibit the typical appearance of small groups and single organisms.

After eighteen or twenty-four hours, the Gram stain shows the colony to contain considerable numbers of autolyzed organisms—a very important diagnostic point. Also after this period the individual colonies enlarge to considerable size, and show many supergrowths,

radial striations, lobated margin, and concentric "oil drop" appearance. N. gonorrhea may be distinguished from Micrococcus catarrhalis by its failure to grow on common infusion agar and its tendency toward early autolysis.

TUBERCULIN: Active Principles of Tuberculin Prepared from Nonprotein Substrates, Eberson, F. Am. Rev. of Tub., May, 1926, xiii, No. 5, p. 454.

A report of the author's studies of tuberculins prepared from nonprotein synthetic media.

The medium was the following:

Ammonium succinate	0.5	gm.
Dipotassium phosphate	0.5	gm.
Magnesium sulphate	0.25	gm.
Calcium chloride	0.135	gm.
Distilled water	100.0	c.c.

To one portion of this substrate 2 per cent glycerine was added. After the tubercle bacilli had been growing on this medium for five to six weeks, the tuberculin was prepared according to the usual method by filtration and concentration to one-tenth of the original volume. The growth and staining properties of tubercle bacilli were modified by this synthetic medium. In the glycerinated portion the microorganisms grew more luxuriantly than in the portion to which no glycerine had been added. In the former, half of the microorganisms lost their original acid-fast characteristic, while all of them lost it in the glycerine-free medium.

From cultures grown on this medium a tuberculin was prepared by fractional alcohol-ether precipitation. The final ether-insoluble fraction represented less than 0.5 per cent by weight of the original tuberculin, contained approximately 4.4 gm. of active substance per c.c. and gave none of the usual tests for protein.

The results of the study may be thus summarized:

Three fractions have been derived by chemical methods for tuberculins prepared with synthetic nonprotein media. They represent alcohol-insoluble, ether-insoluble and ether-soluble substances, and comprise, respectively, 4.6 per cent, 2.3 per cent and approximately 0.5 per cent by weight of the original tuberculin. The ether-soluble fraction is gummy and fatty or waxy, and gives none of the tests for protein.

In the tuberculous guinea pig the potency and specificity of these tuberculin fractions have been demonstrated as early as three days after inoculation of the animal with B. tuberculosis.

It has been shown for the first time that small amounts of tuberculin fractions prepared from protein-free synthetic substrates are capable of sensitizing normal, nontuberculous guinea pigs. In these animals typical skin reactions can be elicited subsequently by intracutaneus injections of minute amounts of homologous as well as heterologous fractions and of unfractionated tuberculin from which such fractions have been prepared.

Clinical trial of these fractions in juvenile patients has demonstrated for the first time that the substances have diagnostic value. Positive skin tests were perfectly correlated with positive clinical and laboratory findings of tuberculous infection. Such was not the case for ordinary old tuberculin in over 20 per cent of young patients in a group of 150 who were studied routinely. In a number of instances the observations suggested that the reactions might be correlated with the degree of activity or with arrested tuberculous infection.

The active substance contained in a dose used for intracutaneous tests in patients was calculated as follows: alcohol-insoluble fraction, 0.005 mg.; ether-insoluble fraction, 0.0025 mg.; ether-soluble fraction, 0.0005 mg.

The present studies point to a method which makes an accurately standardized tuberculin available for routine clinical work. It is believed that the active principle of tuberculin can best be isolated by this or by a similar method.

The observations on sensitization suggest that these may be applied to studies of immunity, and, by extension, to therapeutic studies in the experimental animal. Such work, now in its third year of progress, will be reported in the future.

GRANULOMA: Studies in Coccidioidal Granuloma, III. Mode of Infection, Ahlfeldt, F. E. Arch. Path. and Lab. Med., August, 1926, ii, No. 2, 206.

Experiments dealing with the mode of infection were attempted by rubbing one platinum loopful of the dry mold over an abraded area of the skin, over the mucous membrane of the nose, over the mucous membrane of the mouth and by suspending a platinum loopful in salt solution and injecting it into the trachea through the skin in rabbits and guinea pigs. Also, experiments dealing with the natural mode of infection were attempted by feeding guinea pigs with lettuce contaminated with culture and exposing guinea pigs to air contaminated with broth culture.

Rabbits were more resistant than guinea pigs. All the guinea pigs died spontaneously in from three to four weeks. They maintained their health until the tenth day and then lost in weight quickly. There was a characteristic drop in weight. At necropsy, some of the pigs showed minute nodules in the lungs and liver, some enlarged mesenteric and cervical lymph nodes, some suppurative periorchitis. Microscopically, there were small areas of peribronchial inflammation, edema and hemorrhage in the lung. The liver showed some cloudy swelling; the spleen follicular hyperplasia; the stomach and intestines were normal; the kidney showed a mild acute interstitial nephritis; the lymph glands some hemorrhage, and the brain showed edema. The organism was found in the lung, liver, intestine, lymph gland and spleen.

Guinea pigs fed coccidioides culture on lettuce showed no gross lesions and insignificant microscopic lesions; few adult forms and shells in the intestines and some adult forms in the pharyngeal lymphatics; they lived longer than pigs that breathed culture. The animals that breathed the culture showed deep congestion or hemorrhages in the lungs, and fluid in the pleura; but no more of the adult forms could be found in the lungs than were discoverable in the tissues of the feeding pigs. These differences suggest greater potency of infection by breathing contaminated air than by eating infected lettuce.

As the animals were infected by using the dry mold through the skin, the trachea and the mucous membrane of the mouth, and by exposure to contaminated food and air, it is probable that coccidioidal granuloma may be transmitted through the skin, as well as the respiratory and gastrointestinal tracts.

CARCINOMA: The Quantitative Determination of Albumin by Tannin and Its Use in the Diagnosis of Cancer, Wigand, R. München. med. Wchnschr., March 26, 1926, lxxiii, 521.

Twenty-four test tubes were filled with the same serum diluted in physiologic salt solution in descending geometric progression, so that the first contained a 1:10 dilution, the second a 1:20, the third a 1:40 solution, etc. To each of the tubes, containing 2 c.c. of the dilution, 1 per cent of a limpid, freshly prepared and filtered tannic acid was added. After eight to twelve hours the precipitated albumin was read. The slight veil which rose from the bottom of the higher dilutions was more clearly visible when 2 to 3 drops of a strongly diluted carbolfuchsin solution were added to the tannic acid. The albumin in the first seven tubes settled in dense masses immediately after the addition of the precipitant; in the following 6 to 7 tubes the precipitation lasted several hours, in twenty-four to forty-eight hours even the weakest concentrations showed the flocculation. In this way 0.000001 gm. was determined. This is not possible by any other method.

A series of tests shows that cachectic individuals do not react positively, that the reaction fails in scirrhous carcinoma and after surgical interventions. Medullary carcinomata are the most frequent to be diagnosed. The serum of pregnant women in the first four months is never positive. Syphilis and tuberculosis seem to have equal results.

REVIEWS

Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.

*The Pathology and Treatment of Diabetes Mellitus**

THE first edition of this volume was reviewed in Volume x, page 590, of THE JOURNAL OF LABORATORY AND CLINICAL MEDICINE. This edition has been brought up to date and has many new observations on insulin and on dietary treatment with insulin. All who make any pretense of interest in the problem of diabetes should possess this work.

The Medical Department of the United States Army in the World War Field Operations†

VOLUME EIGHT of the history of the Medical Department of the United States Army in the World War is a description of the organization and the activites of the Medical Department of the American Expeditionary Forces from the arrival of the advance guard until the armistice, with brief histories of the individual combat divisions. The volume is profusely illustrated with U. S. Army official photographs of various medical organizations and institutions in the A. E. F. in France, Italy, Siberia and elsewhere.

It becomes at once evident to the reader that the preparation of this volume has required a tremendous amount of painstaking care. While much of it is bare narrative the human element has not been left out.

The work will naturally find its greatest usefulness as a subject for critical study by army medical officers in this and other countries.

The Thyroid Gland‡

A SMALL volume containing an historical development of the goiter subject and a presentation of the contributions that have been made to our knowledge of etiology, pathology, and treatment in the various departments of the Mayo Clinic.

When we consider that up until 1861 barely a hundred operations had been reported on the thyroid gland, we realize how great has been the progress in the last half century. In 1874 only two surgeons in France, Italy, Great Britain and America had performed more than four lobectomies. In the United States and Canada only forty-five operations for goiter were recorded up to 1883.

*The Pathology and Treatment of Diabetes Mellitus. By George Graham, M.A., M.D., F.R.C.P. Cloth. Illustrated. Pp. 230. Price $2.75. Humphrey Milford, Oxford University Press.

†The Medical Department of the United States Army in the World War. Field Operations. Prepared under the direction of Maj. Gen. M. W. Ireland, The Surgeon General, by Col. Charles Lynch, M.C., Col. Joseph H. Ford, M.C., Lieut. Col. Frank W. Weed, M.C. Cloth. Illustrated. Pp. 1097. Government Printing Office, Washington, D. C. 1925.

‡The Thyroid Gland. By Charles H. Mayo and Henry W. Plummer. Cloth. Pp. 83. The C. V. Mosby Company, St Louis, Mo., 1926.

NOTE: In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

By contrast, when Kocher died in 1917, five thousand cases of goiter had been operated on at his clinic. At the Mayo Clinic from 1912 to 1922 there were twenty thousand, nine hundred and sixty-one resections of the thyroid with six thousand four hundred and ninety-three ligations of thyroid arteries.

Dr. Plummer presents an excellent summary of our knowledge of the function of the thyroid gland. He gives abundant evidence in substantiation of his theory that the symptoms of Graves' disease are due to a dysfunction rather than hyperfunction of the gland. In toxic adenoma we are dealing with an increased secretion of normal thyroxin and the treatment indicated is removal of those tissues which are secreting the excess. In exophthalmic goiter on the other hand there is both a hypersecretion of normal thyroxin and the formation of an abnormal incompletely iodized thyroxin. The latter is responsible for the symptoms of Graves' disease. Here the administration of iodine gives greatest benefit by enabling the thyroid to completely iodize the thyroxin molecule. He presents the record of one patient who, before thyroidectomy, had a basal metabolic rate ranging above plus eighty and since operation a rate of minus fourteen, but with the characteristic nervous phenomena and a progressive exophthalmos still persisting. When this patient is placed on Lugol's solution the nervous phenomena disappear, exophthalmos recedes, the basal metabolism drops to minus twenty-eight and edema, slow speech, etc., characteristic of myxedema appear within two weeks. When iodine is administered and in addition the basal metabolism is maintained at the average normal rate with thyroxin or thyroid extract, all evidence of disease disappears. Here we have a patient who, after operation has a thyroid deficiency requiring the administration of thyroid extract and at the same time is putting out an abnormal thyroxin which requires Lugol's solution for saturation.

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*Practical Dietetics in Health and Disease**

HAVE you, in your experience, had a patient who has had occasion to receive treatment from two different gastroenterologists both of outstanding repute? And then has the task been yours of trying to help straighten him out of the hopeless mental tangle and dismay in which he finds himself after discovering that these two men both equally positive and dogmatic in their statements prescribed quite different diets and that each insists not infrequently that articles of food looked upon with favor by the other are nearly in the class of rank poison?

There is much faddism in dietetics today. Dietetic prescriptions are dogmatically drawn up and rigidly enforced often where there is no sound scientific basis.

Of course, this is often necessary for the purpose of making the patient adhere to a reasonable general dietary.

The volume under review presents a comprehensive list of appropriate diets for a great variety of disorders. The different diseases are arranged alphabetically, thus facilitating the physician's finding the appropriate dietary recipe once his diagnosis has been established. Most of the diets are of a dogmatic type but they are distinctly useful in that they are something which the physician can give to his patient and which the patient can follow without difficulty.

The author makes no attempt to provide a comprehensive discussion of the dietary principles in those diseases in which diet is of the greatest importance such as food allergy, nephritis, diabetes, pellagra, ulcer, pernicious anemia. Appropriate dietaries for individual instances of these diseases are included.

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*Practical Dietetics for Adults and Children in Health and Disease. By Sanford Blum, A.B., M.S., M.D. Cloth. Pp. 362. Price $4.00. F. A. Davis Company, Publishers, 1926.

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EDITORIALS

Ring and Flocculation Tests in Tuberculosis

A CERTAIN amount of attention is being given at present to a number of serum precipitation (flocculation and ring) tests which have been introduced in the hope of giving diagnostic and prognostic information in tuberculosis. The tests which have been employed most in this country are Daranyi's,[1, 2, 3] a flocculation test performed with alcohol-salt solution, which has been studied by many including Enderle,[4] Pinner,[5] and Baum and Schumann[6, 7] who have modified the technic somewhat with a view to making the test more quantitative; the tests of Larson and his associates,[8, 9, 10, 11, 12] one of which involves the use of an extract of tubercle bacilli, the other a 0.2 per cent solution of tricresol in 0.85 per cent sodium chloride solution; and a semisecret commercial preparation known as "Tubercumet,"[12, 13, 14] to which we shall revert later.

In Europe a greater variety of these tests have been reported. Setting aside earlier studies for the moment, the tests now appearing in the literature

are Daranyi's, Matefy's,[15, 16] 0.05 per cent aluminum sulphate; Lange and Heuer's,[16, 17] a derivative of the simple distilled water test attributed to Klausner, distilled water and 1 per cent silver nitrate plus light; Mündel's,[18] 18 to 19 per cent ammonium sulphate; Bonaeorsi's[19, 20] a derivative of Dold and Sachs-Georgi tests; and a test bearing the name of Fornet,[21] which is apparently being exploited commercially. The studies of Vernes and Prunell[35] with 1.25 per cent resorcin; of Sachs and Oettingen with heat, alcohol, sodium chloride, and ammonium sulphate; of Frish and Starlinger with saturated chloride solution; and of Sachs and Klopstock[32] with lecithin and calcium chloride, should also be mentioned.

It will be noted that in nearly all these tests where the composition of the preparation is given with scientific candor, the solution contains some substance which is known to precipitate globulin within suitable ranges of concentration. Wherever the biochemistry has been seriously investigated the precipitate is believed to be due to increased colloid lability, which in turn is associated with increased serum globulin, perhaps in combination with other physicochemical factors such as variations in the amount of lipoids present, and the hydrogen-ion concentration.

In 1910 Porter[22] made a very interesting and significant study of precipitation tests. He tested sera from several hundred persons by the following method: Three solutions were prepared, (a) Bacillary emulsion diluted 1:50, made isotonic (0.85 per cent NaCl), and filtered through porcelain; (b) The same with the addition of 0.5 per cent phenol; (c) 0.5 phenol in 0.85 per cent salt solution, without bacillary extract. Each of these solutions was mixed with an equal amount of serum diluted 1:20, and incubated twelve hours at 37° C. Positive results, which varied from a slight sediment to a marked precipitate in suspension, were secured in about 85 per cent of all tuberculous sera; the percentage was lowest in very advanced cases. About 30 per cent of sera from nontuberculous persons gave positive results, but this group included many patients with other diseases. These figures compare pretty favorably with most of those reported in subsequent studies on precipitation tests, though in some of the literature cases with doubtful diagnoses have obviously been grouped according to the result of the test, thus giving a false impression of great accuracy. The most interesting point is that Porter got almost identical results with all three solutions employed, and concluded that for practical purposes the phenol-salt solution alone was satisfactory. He calls attention to the fact that Stoerck,[23] in the preceding year (1909) had noted the precipitation of tuberculous sera by phenol. This reaction is now familiar and quite certainly explained by globulin increase and increased "colloid lability." But the cause of the precipitation with the isotonic 1:50 bacillary extract remains a problem, and a very interesting one.

Calmette[24] in his encyclopedie "L'Infection Bacillaire et la Tuberculose" reviews previous work on precipitation tests including studies of his own with Massol, first reported in 1910. Calmette and Massol[25] concluded that there was no evidence of a specific reaction between the dilute tuberculins

used and any component of tuberculous serum. Precipitates sometimes resulted, but these precipitates contained no tuberculin, and did not reduce the strength of the tuberculin used. They observed similar precipitation on diluting the sera with five volumes of distilled water, and attributed the positive results with tuberculin to dilution of the sera, implying that the tuberculins were diluted with distilled water.

Calmette cites Porter's work but unfortunately quotes his percentages incorrectly, and does not mention the precipitation by phenol salt solution alone, nor the important point that Porter's dilute tuberculin without phenol was isotonic, so that there can be no question in that case of a simple precipitation of globulin in hypotonic solution.

There is a large recent literature on the precipitation tests, dealing chiefly with the percentage of accuracy in known clinical conditions. The figures vary with different tests and in different reports, but there is pretty general agreement on the following points:

1. The percentage of positive tests is very low in health.

2. The percentage of positive tests is high in active tuberculosis.

3. Tuberculous persons giving negative tests are most of them either extremely sick or virtually free from symptoms. Agreement on this point, however, is not complete.

4. Any of the tests may be positive in a variety of conditions other than tuberculosis. They seem to be commonly positive in acute respiratory infections.

5. A repeatedly positive test in the absence of other infectious disease may be of assistance in confirming a doubtful diagnosis of tuberculosis. The tests may be of some use in prognosis, but they do not seem likely to replace good clinical judgment, or even to be of very great assistance to it.

It would seem wise, instead of multiplying these procedures indefinitely, to concentrate on finding out what they depend upon. As already stated, the reagents in most of them are known to contain substances which precipitate globulins under suitable conditions of concentration, influenced perhaps by the state of acid-base equilibrium. Of three methods to which this statement does not obviously apply, Bonacorsi's depends upon a cholesterolized alcoholic extract of tubercle bacilli, diluted with physiologic salt solution. It is said to give a considerable percentage of agreement with the Sachs-Georgi reaction, which in turn appears to be a nonspecific test for globulin lability. The tubercle bacillus extract is not an essential part of Bonacorsi's solution, according to Kovats.

In 1921 Fornet[33, 34] reported an agglutination test performed with tubercle bacilli defatted with ether vapor at 40°. The technic of the test is not given in full. The "Fornet" diagnostic preparation, apparently sold as an agglutination test, is said by Bignami to be "a 0.6 per cent carbolic solution of sodium phosphate" containing some more or less acid-fast tubercle bacilli, and these are said to be already partially agglutinated.[32] Bignami attributes positive results to precipitation of globulin in excess by the acidity of the solution, but the carbolic acid alone is sufficient. Larson and Montank,

continuing the observations of Stoerck and Porter, have shown that various phenols, cresols, and other chemicals give typical ring tests.

"Tubercumet," according to Boissevain and Ryder of the Colorado Foundation, contains a considerable amount of phenol and only a minute amount of nitrogen, yet it gives heavy rings in many cases. The test is probably in essence another phenol test for globulin; certainly in the presence of phenol any other factor cannot be evaluated. Henry and Hatch have published an indefinite account of the preparation of a fractional extract of tubercle bacilli in the manufacture of Tubercumet, but they do not give the chemical composition of the solution in which this extract is dissolved.

The status of these tests seems similar to that of the red-cell sedimentation test, which is not an immunologic phenomenon in the limited sense, but depends on quantitative changes in a physicochemical system, apparently involving a relative increase of globulin with reduction of the surface tension of the plasma. When a number of precipitation tests are done on the same bloods they do not agree in all cases with one another, nor with the sedimentation test; but all tend to be positive in the same class of conditions. Schanin and Chrennikow[26] in a study of seventy cases of extrapulmonary tuberculosis, found a distinct correlation between globulin increase, reduced surface tension, reduced electric conductivity and dissociation, and increased sedimentation rate; these in turn were correlated with a rise in the isoelectric point and precipitation within wider range of P_H. That is, when 0.01 N lactic acid is added to the serum, precipitation begins nearer neutrality and continues to a higher acidity than is the case with normal sera.

Lehmann-Facius,[27] titrating syphilitic sera with 1:1000 lactic acid, found that in Kahn-positive sera precipitation began with less acid than in normals but the range was not increased, while Sachs-Georgi-positive sera precipitated with less acid than normals and also continued to precipitate to a higher acidity, indicating increased total globulin. He considers that both the amount of globulin and the amount of available ionized hydrogen are concerned in the flocculation. It would seem that the alkali reserve and buffer salts must also have a direct effect on these titrations.

Further study is very desirable with a view to comparing a number of the precipitation tests for tuberculosis and correlating them with the actual globulin content, which is known to be commonly increased in tuberculosis, though it may be diminished in cachexia;[21, 26, 28] and it is noteworthy that very advanced cases doing badly often give negative precipitation tests. It may be that all the tests in common clinical use depend on globulin increase solely, in which case it should be possible to select one and place it on a more or less quantitative basis. The work of Daranyi and Baum and Schumann shows a tendency in this direction, though the alcohol-salt solution test may not be the best possible.

On the other hand the relation between globulin concentration and precipitation may be more complex. Reports in general certainly indicate that the flocculation tests commonly used in the diagnosis of syphilis have at least relative specificity, as compared with a simple globulin precipitation test like that with ammonium sulphate, with which Mündel got 100 per cent positive reactions on syphilitic children and 98 per cent on tuberculous children.

Apart from the tests known to depend on simple chemical solutions there remain those in which the reaction, involving an extract of tubercle bacilli, may possibly be an antigen-antibody one. Some of the reports of tests using bacillary extracts are impossible to interpret because the authors do not explicitly state that the extract was made with simple physiologic salt solution;[29] while in other instances, such as Hollaender's,[30] it is stated that phenol is used. On the other hand there are the experiments of Porter, already described; and in 1923 Larson, Montank, and Nelson[8] reported about 200 cases tested with antigen solutions prepared by disrupting tubercle bacilli with liquid carbon dioxide and filtering. The results showed a fairly high percentage of accuracy. Antigen solutions prepared from acid-fast actinomyces, however, gave positive results corresponding to the tubercle bacillus antigens, while those prepared from non-acid-fast actinomyces gave negatives, as did broth filtrates from both tubercle bacilli and actinomyces. These antigens were dissolved in physiologic salt solution, and Dr. Larson[31] is quoted as considering that "the results with blown bacilli seemed to be more specific than with the phenol compounds." A series of tests like Porter's comparing Larson's two methods, one containing bacillary extract but no phenol, the other phenol (trieresol) and no tubercle bacillus extract, both in physiologic salt solution, would be of more interest than most of the studies on precipitation tests in tuberculosis which have appeared as yet. It would be well also to pursue the idea of Calmette and Massol, and try to determine what changes occur in the antigen solution and also in serum as the result of the formation of a precipitate.

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—*G. B. W. (C. T. R.)*

The Reactions of the Omentum

THE apparent facility with which the omentum finds its way to an inflamed area or a foreign body in almost any location within the abdominal cavity has led many to believe that the mechanism of this movement must be a purposeful one rather than dependent entirely upon hazard. Some of the terms applied to the omentum give voice to this impression. Thus we read of the abdominal policeman, the friend in need, the great leucocyte.

Some have attributed to it a power of intrinsic movement. They believe that it has special stretching and contractile capacity. Rutherford Morrison states in his *Introduction to Surgery* that the omentum travels around the abdomen with considerable activity and is attracted by some sort of information to neighborhoods in which mischief is brewing. Saint[1] explains the movement as a phenomenon of chemotaxis. Norris,[2] however, has shown that the omentum contains no muscle fibers and possesses no inherent motile power.

The weight of evidence at present would indicate that the movement of the omentum to an inflamed area is purely passive. Adam,[3] Wilkie,[4] and Durham[5] believe that the omentum is carried along chiefly by intestinal peristalsis.

Florey and Carleton[6] present a convincing series of experiments corroborating the theory of passive movement. Mice and decerebrate cats kept immobilized in the dorsal position for periods of from one hour to two days after the beginning of the experiment, were given intraperitoneal injections of carmine pigment. They then found that only the portions of the omentum which dipped down into the side of the abdominal cavity contained carmine granules. The omenta of control animals which were allowed to resume their normal activities after the injections succeeded in collecting nearly all of the material. Similar results followed the insertion of large foreign bodies such as pith and cotton wool into the abdominal cavity.

Experiments with living bacteria on immobilized decerebrate cats, in which abscesses were produced in the intestinal walls and on the liver surface showed that here again no evident attempt was made by the omentum to reach the areas of inflammation. Cotton wool soaked in a culture of Staphylococcus albus was placed in the closed end of a glass tube. The open end of the tube was inserted into the abdominal cavity. The omentum made no attempt to reach the infected foreign body through the open tube. Chemotaxis apparently played no part.

From observation through a window inserted in the abdominal wall the authors concluded that the posture was probably the main determining cause of the apparent movement of the omentum to sources of infection. By bending

the cat they observed that a selected portion of the omentum could be made to alter its position by as much as three centimeters. In this way it could be moved into various positions. Diaphragmatic excursion appeared to play little part. Peristalsis moved parts of the omentum and it would be possible to account for its rolling up around foreign bodies by movement imparted by peristalsis.

The omenta of a cat and a rabbit delivered from the abdominal cavity and observed in a glass cell, with blood supply undisturbed, made no attempt to envelop a piece of cotton wool in contact with them or a piece of cottton wool soaked in a culture of Staphylococcus albus placed two or three millimeters from them. The observation extended over a period of sixteen hours. Negative results were likewise obtained with the excised omentum in vitro.

The evidence against intrinsic movement of the omentum appears quite conclusive. And yet it is remarkable with what facility the omentum becomes attached to the site of a local inflammation or to a foreign body. Peristalsis probably plays a part but body movement appears to be the most important factor. It has been remarked that the bent posture of one suffering with severe abdominal pain facilitates the omentum reaching well down into the pelvis. Twisting and squirming movements likewise appear to be not without logical reason.

Once the organ has established contact, the pathologic picture is distinct. At the point of contact a fibrin exudate forms and within an hour or so quite firmly anchors the omentum to the point of pathologic change. Soon leucocytes migrate into the fibrin layer and where pigment granules are present they actively engulf them. The fibrin clot later becomes organized by fibroblasts and new vessels grow out into the recently organized tissue. At the end of from three to five days the site has often become highly vascularized. As a last stage collagen fibers are produced by the fibroblasts and the damaged area or foreign body becomes encapsulated. With the disappearance of fibrin and the epithelialization of the marginal contact surfaces the reaction is completed.

The heavy vascularization of omental adhesions is not infrequently of great value. An outstanding example is in the providing of collateral circulation in cirrhosis of the liver.

LePlay[7] found that even the resected omentum, when left in the peritoneal cavity, retains the power to react to foreign bodies.

Recently the possibility of a chemotactic factor has been again raised, although in a somewhat different form. Sanarelli[8] has studied a curious phenomenon which he designates as galvanotropism. He, like others, has observed the rapid engulfing of particles of carmine or charcoal or lycopodium spores by the omentum. He has found that cholera vibrios pass rapidly into the omentum where they accumulate and agglutinate. He, also, discards in great part the theory of intestinal movement or intraperitoneal currents and suggests that the contact of the omentum with a foreign body, such as bacteria, may be dependent on some physicochemical process, a colloidal reaction between the peritoneal serosa acting as gel and a microbe or powder which, suspended in the fluid, is charged electrically. He recalls that bac-

teria, colloidal granules, and infusorial substances possess an electric charge varying according to the nature of the material. Indeed a recently suggested classification of bacteria is based on the electrical reactions of the various microorganisms.

Sanarelli observed that if an omentum is removed from a rabbit or guinea pig and placed in distilled water and then is touched with a clean platinum needle, the organ adheres to the platinum with considerable force. Once rolled about the needle it is separated from it with considerable difficulty. He believes that the omentum charged positively is attracted to the platinum which carries a negative charge. The omentum being a colloidal membrane follows the laws of colloids. If a surface colloidal membrane, chemically inert, comes in contact with a mineral salt dissociated in water into its respective ions, the membrane combines with the latter to form a complex colloid. Thus, the colloidal gel becomes chemically active acquiring the qualities and properties of ions.

The surface of the omentum normally carries a positive electric charge. This can be neutralized by prolonged immersion in sodium chloride solution or in dilute hydrochloric acid after which the omentum no longer adheres to a platinum wire. The reaction is, however, reversible and the power of adhesion will return after prolonged immersion in distilled water. Sanarelli observed a similar reaction in a study of lymph glands but none with such tissues as muscle, liver, kidney and spleen.

The phenomenon is of interest. The author's hypothesis may be correct. Its application as an explanation of the clinical processes under consideration will of course require substantiation.

Omental function is not limited to the removal of foreign bodies from the peritoneal cavity or to its protective adhesion to areas of inflammation. The omentum is active in the absorption of fluids from the abdominal cavity. Wilkie[4] found that salt solution introduced into the abdomen of animals whose omentum was intact was absorbed half again more rapidly than in animals in whom the organ had been removed. The omentum possesses distinct bactericidal properties. It contains large numbers of phagocytes both scattered through it and in nodal accumulations along the blood vessels. Wilkie injected broth cultures and found that the omentum still contained viable organisms after the peritoneal cavity itself had become sterile. Portis[9] has attempted to show that the omentum assists in antibody production. There is some evidence that this is the case in the rabbit but he concluded that the evidence in the dog and guinea pig was decidedly less convincing.

The omentum aids in the vascularization of tumors and of organs damaged by infarct. Mechanically, it probably serves also a function somewhat analogous to a ball bearing, in that it interposes a lubricated movable system between the intestines and the parietal peritoneum.

It contains a network of freely anastomosing blood vessels without a large capillary bed. Rich[10] has, however, demonstrated the existence of a fairly extensive capillary bed in the omentum of animals whose capillaries have been dilated following histamine shock. The larger blood vessels are surrounded by layers of fatty tissue. The thinner portion consists of cells

enmeshed in a mass of interlacing, connective tissue fibrils and covered by flattened mesothelial cells. In the rabbit we find a special structure, the *taches laiteuses,* aggregations of various types of free cells, in close contact with areas of increased vascularity. In the guinea pig the *taches laiteuses* are replaced by glomerulus-like bodies with characteristic central capillary networks and peripheral cellular aggregations. In the dog there is neither *tache laiteuse* nor glomerulus-like body. The vessels form extensive anastomoses throughout and there are small cellular aggregations of phagocytes along the vessels. Both the *tache laiteuse* of the rabbit and the glomerulus-like body of the guinea pig are characterized by their abundant blood supply, each being provided with capillary tufts with afferent and efferent vessels. The glomerulus-like bodies usually have two afferent terminal arteries with one efferent vein and a rather extensive intervening capillary network.

The various types of cells observed in the omentum have received close study. For many years it was thought that the fibroblasts and the serosal lining cells were interchangeable. It was thought that as the mesothelial cells were shed in acute inflammation their places were taken by fibroblasts which eventually formed new serosal cells. Kiyono was perhaps the first to deny the supposition. Cunningham[11] has shown apparently conclusively that the fibroblasts and the serosal cells are distinct and always remain so.

A third type of cell found in the omentum is the leucocyte and a fourth, the clasmatocyte. This latter resembles the leucocyte in general appearance but possesses dendritic processes which for a time led to the belief that they were derived from the fibroblasts. Maxinow,[12] however, demonstrated that the clasmatocyte is a distinct type of cell. He introduced two sterile cover slips under the skin of a rabbit and determined the speed with which various types of cells passed between the covers. The leucocytes appeared first, then the clasmatocytes which wandered in after about nineteen hours. These were followed later by the fibroblasts. Furthermore the clasmatocyte is especially sensitive to certain dyes, particularly neutral red. It appears to be a cell of connective tissue origin, specifically differentiated to take up and store particulate matter. Apparently it originates primarily from endothelium.

Portis[9] injected India ink into the peritoneal cavities of rabbits and removed the omentum after twenty-four hours. On microscopic study the ink was found contained chiefly within large phagocytic cells, most numerous in the *taches laiteuses.* Similar results were obtained with a 5 per cent suspension of carmine. The results were the same after the injection of chicken red blood corpuscles, whose nuclei could be clearly identified. By simultaneous intravital staining with trypan blue the author demonstrated that the leucocytes and particularly the clasmatocytes were the active cells phagocytizing the foreign particulate matter.

Portis found that twelve hours after the injection of 10 c.c. of a mixture containing 5 c.c. of defibrinated chicken blood and 5 c.c. of a 25 per cent acacia suspension, in a rabbit, the fluid was practically all absorbed but that many smaller and larger clumps were adherent to the omentum. Stained preparations showed many chicken red cells adherent to the external surface of the

omentum and a few intact erythrocytes contained within the vitally stained phagocytic cells in the *taches laiteuses*.

After twenty-four hours these latter cells were loaded with the nuclei from the chicken corpuscles while the cell bodies of the corpuscles could no longer be recognized. After forty-eight hours there was considerable fragmentation of these nuclei so that they were almost unrecognizable. Finally, at the end of ninety-six hours only a considerable amount of granular material could be found in the cytoplasm of the cells.

It is of interest that in rabbits who had twelve days previously received 5 c.c. of defibrinated chicken blood, this whole process was speeded up so that at the end of six hours the clasmatocytes were found loaded with chicken corpuscle nuclei, and the granules had nearly disappeared by the end of twenty-four hours. These previously immunized rabbits were found to take the trypan blue vital stains more extensively and there appeared to be more clasmatocytes present than in the controls.

This increased response after previous sensitization was by no means as pronounced in guinea pigs as in rabbits. The results correspond with the lessened ability of the guinea pig to produce antibodies.

Vaughan[13] has shown that the local inflammatory reaction to bacteria, once they have penetrated the omental tissues, does not differ in any important respect from similar reactions in other tissues of the body. Bacteria may penetrate the peritoneum elsewhere than in the omentum, but this appears unusual. Probably the greater motility of this organ and the flexibility of its covering are factors.

The omentum is an abdominal organ of greatest importance, and yet surprisingly little reference is made to it in the standard works on pathology. Indeed the surgeon on the whole may be said to know more of the pathology of this tissue than does the pathologist. The possibilities for productive studies on the omentum should be particularly alluring to the immunologist.

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—*W. T. V.*

Cults and Their Relation to Medical Practice

AS LONG as credulity, ignorance, and unscrupulousness are human attributes cults and irregular, not to say weird and absurd, methods for the treatment of disease will arise and flourish for a time to give their place to another offspring of absurdity and sesquipedalianism.

Were it not for the regrettable and often disastrous aftermath so frequently visited upon the victims of charlatanry there would be little reason to dignify these fads and fancies by comment or discussion.

Inasmuch, however, as the compelling interest of the medical profession now lies as much in the evolution of means for the prevention as well as the treatment of disease, and as the achievement of both is closely related to the measure of their general and public understanding, some consideration of cults in their relation to medical practice is necessary in any consideration of the public health.

Practically all irregular methods of treating disease are founded and thrive upon the fact that the average man or woman has only the haziest idea of the functions of the human machine and the mechanism whereby they are performed. It is true that there are courses, so-called, in physiology in the schools, and it is equally true that for every ounce of information in the possession of the average adult, there is a pound of misinformation to more than counter-balance.

It matters not how skilled the individual in the arts or sciences; how learned in the higher mathematics or in the mazes of law or philosophy, let him be sick and the most amazing abysses of misconception are often brought to light.

Physicians are often amused and sometimes astonished to find a patient otherwise well-informed and intelligent who gives a history of treatment by various irregular and sometimes absurd methods, for the subjects of the oscilloclast, even as the by-gone champions of the Perkins' tractors, are by no means invariably of the "ignorant classes." One often hears astonishment at the credulity so displayed but seldom is there any evidence of appreciation of the relation of the doctor to, or his degree of responsibility for, the situation as it exists.

The average human being pays very little attention to his bodily mechanism as long as it functions without undue friction, and the average individual is seldom "sick" until his disabilities and functional disturbances have reached a stage where they interfere with his normal and accustomed habits. Then he seeks the doctor, and the same man who hesitates twice and thinks thrice before selecting his broker, banker, lawyer or even his tailor, very often steps blithely through the first door labeled "Doctor."

This is not as astonishing as at first glance it appears. The client first visiting a lawyer does not expect an immediate decision. Authorities must

be consulted; the individual, and, be it said, to the lay mind the sometimes incomprehensible, pronunciamentos ore rotundo of learned judges, upon which the law and its appurtenances seem founded must be compared; and the more numerous the decisions cited, the more gratified is the client at the study given to his problem.

Even the garage mechanic is given a day or so for overhauling before the extent of the necessary repairs becomes known.

But the Doctor! Picture the doctor requesting his patient to wait while authorities are consulted!

In the ancient days, when the doctor and the high priest were often one; when diseases were visitations of the gods, divination and supernatural omniscience were expected of the physician, and found.

There are few men practicing who may not find in their offices patients "making the rounds." How many histories disclose a similar tale: A recital of the complaint; a snap diagnosis, "biliousness," "indigestion," various "conditions," a handful of pills; a prescription. Then, another doctor; a pooh-pooh for the previous diagnosis; another diagnosis; another prescription; some different pills, and, again, another doctor and the same story.

How many patients there are who are not only delighted but astonished when a thorough preliminary examination is insisted upon, laboratory studies inaugurated, and a frank statement made of the necessity for study, and who voice surprise that no such studies were made before.

Consider the patient trudging from office to office and accumulating such diagnoses as "thin blood; sluggish liver; stomach full of gas; chronic appendicitis" and so on, and so on.

Consider the tabetics operated upon for gallstones; the pneumonias for appendicitis; the diabetics discovered *after* operation—and all for lack of preliminary study.

The patient sees no incongruity in telling his story, advancing *his* opinion as to what is the matter with him and, within a few moments, hearing the diagnosis. His innate belief in the omniscience of the doctor is thus bulwarked by his concrete experience and there is little to marvel at if, later, he receives with equal faith the pronunciamento of this, that, or the other "doctor," of absurdity.

The pill-peddling physician; the careless physician; the snap diagnostician; the pseudoscientist who probes the depths of pathology with a single blood count or urinalysis, are all responsible for perpetuating if not encouraging lay ignorance and thus serve as more or less direct feeders to cults and irregular practitioners because of their failure to enlighten the patient or to disabuse his mind of the idea that an immediate diagnosis may be, at times, impossible.

Credulity and ignorance go hand in hand and it is the privilege as well as the duty of the physician to destroy both.

If every patient were first thoroughly studied and the necessity for the study made clear; if the disturbed functions were brought to light and the mechanism explained; if a definite effort were made to make intelligible to

every patient the nature of his illness and the relation it bears to his symptoms as well as the rationale of the remedial measures to be employed, by so much would ignorance be dispelled and credulity destroyed.

There is crying need for the education of the patient by the physician.

—*R. A. K.*

*A New Method of Examining Fresh Tissue**

ANY laboratory which needs a quick, inexpensive, simple method for preparing fixed or fresh tissue for microscopic examination, will find Terry's "razor section" method with the recent modifications of this stain a valuable aid.†

The technic first used by Terry in the preparation of tissue consisted in cutting thin flat slabs of fixed tissue with a razor, staining one side of the section with polychrome methylene blue and using strong reflected light for illumination. The details were surprisingly clear. Cells were well stained and under low magnification nuclear details were plainly visible. In fact one familiar with the method could decide whether a lesion was benign or malignant. It makes possible the examination of a large amount of material in a short time and if it becomes necessary to resort to paraffin or frozen sections the same pieces of tissue can be used.

With the modified technic sections of either fresh or fixed tissue may be utilized. Since only one side of the section is stained and only the stained cells absorb the light to any appreciable extent, the sections are now examined by transmitted light in the usual way. The source of light is an ordinary frosted Mazda electric light bulb. Transmitted light is more satisfactory than reflected light as higher magnification is possible with it. Also the frosted bulb is much cheaper than the lamp formerly used. The preparations are so clear that the characteristics of cancer cells are easily made out.‡

The method can be applied to numerous procedures. It can of course be used in the practice of surgical pathology or in postmortem work. If large pieces of tissue are to be examined many areas can be studied in a short time. The criticism of the freezing of tissue for sectioning has been overcome by Terry's technic.

The method has certain limitations; preparations are not permanent; they must be examined within a few minutes after they are made and the tissue must be of sufficient consistency to allow a flat surface to be cut with a razor.

—*H. D. C.*

*A new principle in the microscopic examination of fresh unfixed tissue. Proceedings of Weekly Staff Meeting of Mayo Clinic, 1926, i, 209.

†The details of the technic and of the preparation of the stain were presented at the meeting of the American Socity of Clinical Pathologists at Washington, May, 1927.

‡MacCarty, W. C.: The Cancer Cell and Nature's Defensive Mechanism Against It. Surg., Gynec. and Obst., 1925, xli, 783-793.

Errata

In the May issue, article by McGuigan, "The Pharmacology of Iron and Aluminum in Relation to Therapeutic Uses," the last sentence in line 20, p. 792, should read:

According to this aluminum is ten times less toxic than ferrous sulphate, which is much less toxic than ferric salts.

In the June, 1927, issue of the JOURNAL OF LABORATORY AND CLINICAL MEDICINE, in the article by Dr. S. L. Leiboff entitled, "A Note on the Measurement of Blood for Chemical Examination," the last two lines on page 912 are transposed.

In the June issue of the Journal, in the article by R. B. Barton, "The Endothelioid Cell in Acute Leucemia," the following corrections are noted in the text:

On page 856, line 31—23.5 per cent should be 25.5 per cent.
In line 32—46 per cent should be 49 per cent.

In the Table on page 857—in the first blood count:
Plasma cells should be 0.4% instead of 0.2%, and the Neutrophilic myelocytes should be 8.8% instead of 4.8%.

In the second blood count:
Myeloblasts should be 25.5% instead of 45.5%.